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(54) Title: SELECTIVELY N-ALKYLATED PEPTIDOMIMETIC COMBINATORIAL LIBRARIES AND COMPOUNDS THEREIN			
(57) Abstract			
<p>The instant invention is directed to a single, selectively N-alkylated compound and libraries of such compounds as set forth in Formula (I). Furthermore, the instant invention is directed to methods of effecting analgesia, a decrease in the postprandial rise in the blood glucose levels of a mammal after ingestion of a carbohydrate load by said mammal, and treating microbial infections, utilizing such a single compound of Formula (I) in conjunction with a pharmaceutically-acceptable carrier. Also, the instant invention is directed to methods for selective alkylation, positional scanning and iterative synthetic and screening technologies.</p>			

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**SELECTIVELY N-ALKYLATED PEPTIDOMIMETIC
COMBINATORIAL LIBRARIES AND COMPOUNDS THEREIN**

This application claims the benefit of U.S. Provisional Application No. 60/____, filed March 5, 5 1996, which was converted from U.S. Serial No. 08/611,390, and is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to novel, selectively N-alkylated compounds of Formula I 10 below, as well as novel libraries composed of many such compounds, methods of synthesizing and screening the libraries, and methods of using the compounds.

BACKGROUND INFORMATION

The process of discovering new therapeutically 15 active compounds for a given indication involves the screening of all compounds from available compound collections. From the compounds tested, one or more structure(s) is selected as a promising lead. A large number of related analogs are then synthesized in order 20 to develop a structure-activity relationship and select one or more optimal compounds. With traditional one-at-a-time synthesis and biological testing of analogs, this optimization process is long and labor intensive. Adding significant numbers of new structures to the compound 25 collections used in the initial screening step of the discovery and optimization process cannot be accomplished with traditional one-at-a-time synthesis methods, except

over a time frame of months or even years. Faster methods are needed that allow for the preparation of up to thousands of related compounds in a matter of days or a few weeks. This need is particularly evident when it 5 comes to synthesizing more complex compounds, such as the instant compounds composed of two or more monomers, each monomer possessing more than one variable substituent.

Solid-phase techniques for the synthesis of peptides have been extensively developed and 10 combinatorial libraries of peptides have been generated with great success. During the past four years there has been substantial development of chemically synthesized combinatorial libraries (SCLs) made up of peptides. The preparation and use of synthetic peptide combinatorial 15 libraries has been described, for example, by Dooley in U.S. Patent 5,367,053, Huebner in U.S. Patent 5,182,366, Appel et al. in WO PCT 92/09300, Geysen in published European Patent Application 0 138 855 and Pirrung in U.S. Patent 5,143,853. Such SCLs provide the efficient 20 synthesis of an extraordinary number of various peptides in such libraries and the rapid screening of the library which identifies lead pharmaceutical peptides.

Substituent limitations have been overcome for mixtures of peptides and peptidomimetics through the use 25 of solid phase techniques instead of the more traditional solution-phase ones. An important step in the development of solid-phase techniques was the discovery of methods to identify active individual compounds from soluble mixtures of large numbers of compounds, as

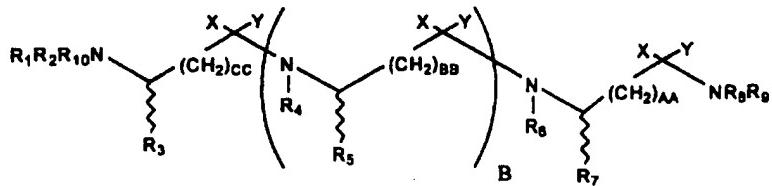
described, for example by Rutter in U.S. Patent 5,010,175 and Simon in WO PCT 91/19735. These soluble mixtures, however, have never before been applied to compounds with amide backbones that have different substituent on each 5 amide nitrogen. Until now, it was possible by previously known methods to add only the same specific substituent to each and every nitrogen atom of the amide backbone. Thus, improved methods were needed to synthesize such selectively N-alkylated amide compounds.

10 This invention satisfies these needs and provides related advantages as well. The present invention overcomes the known limitations to the shortcomings of combinatorial chemistry with respect to selective N-alkylation. The present invention combines 15 the techniques of solid-phase synthesis of peptidomimetic compounds and the general techniques of synthesis of combinatorial libraries to prepare new selective N-alkylated compounds.

SUMMARY OF THE INVENTION

20 This invention is directed to a single selectively N-alkylated compound or a library of an approximately equimolar mixture of two more selectively N-alkylated compounds of the Formula (I):

(I)



Wherein:

R₁ and R₂ independently are a hydrogen atom, an
 5 amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀
 cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂
 substituted alkyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted
 alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted
 C₆ to C₁₅ alkyl heterocycle;

10 R₃, R₅, and R, are independently a hydrogen atom,
 C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl,
 substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆
 substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a
 substituted C₆ to C₁₅ alkyl heterocycle;

15 R₄, R₆, and R₈ are independently a C₁ to C₁₈
 substituent group; with the proviso that all but one of
 R₄, R₆ and R₈ can simultaneously be the same group;

R₉ is a hydrogen atom or a solid support;

18 R₁₀ is optionally present as a C₁ to C₁₈
 20 substituent group when R₁ and R₂ are other than a hydrogen

atom, an amino protecting group or when both R₁ and R₂ are C₁ to C₁₂ acyl groups;

AA, BB, and CC are independently 0 to 5;

B is from 0 to 3;

5 further wherein the stereochemistry at the carbons bonded to R₃, R₅, and R₇ are independently R or S or a mixture of the two;

further wherein when B is 2 or 3; each R₄ and R₅ can be the same or different;

10 with the proviso that either R₁ or R₂ can be taken with R₃; R₄ can be taken with R₅; and R₆ can be taken with R₇; respectively and independently, to form a substituted or unsubstituted pyrrolidine ring;

X and Y are either 1) each a hydrogen atom or
15 2) taken together to represent a carbonyl group;

and a pharmaceutically acceptable salt, solvate or hydrate thereof.

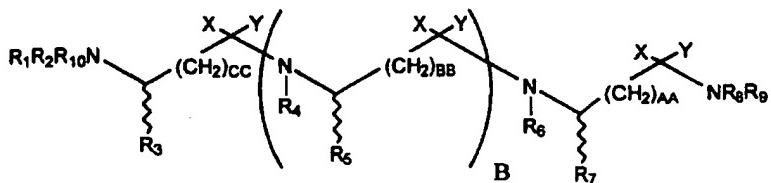
This invention is also directed to iterative and positional scanning methods of synthesizing the
20 libraries of compounds described above as discussed below. Another aspect of the invention is a method of selective N-alkylation as set forth below. Furthermore, the invention comprises methods for affecting analgesia

in a mammal, effecting a decrease in the postprandial rise in the blood glucose levels of a mammal after said mammal has ingested a carbohydrate load, and a method for treating microbial infections, all of which methods 5 comprise administering a single compound of the above formula in conjunction with a pharmaceutically acceptable carrier, as set forth below.

DETAILED DESCRIPTION OF INVENTION

The instant invention is directed to a single 10 compound or an approximately equimolar mixture of two or more selectively N-alkylated compounds of the Formula (I) :

(I)



Wherein:

15 R_1 and R_2 independently are a hydrogen atom, an amino protecting group, C_1 to C_{12} acyl, C_3 to C_{10} cycloalkyl, C_3 to C_6 heterocycle, C_1 to C_{12} alkyl, C_1 to C_{12} substituted alkyl, C_7 to C_{16} alkylaryl, C_7 to C_{16} substituted alkylaryl, a C_6 to C_{15} alkyl heterocycle, or a substituted 20 C_6 to C_{15} alkyl heterocycle;

R_3 , R_5 , and R_7 are independently a hydrogen atom, C_1 to C_{12} alkyl, C_1 to C_{12} substituted alkyl, phenyl,

substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₄, R₆, and R₈ are independently a C₁ to C₁₆ substituent group; with the proviso that all but one of R₄, R₆ and R₈ can simultaneously be the same group;

R₉ is a hydrogen atom or a solid support;

R₁₀ is optionally present as a C₁ to C₁₆ substituent group when R₁ and R₂ are other than a hydrogen atom or an amino protecting group;

AA, BB, and CC are independently 0 to 5;

B is from 0 to 3;

further wherein the stereochemistry at the carbons bonded to R₃, R₅, and R₇ are independently R or S or a mixture of the two;

further wherein when B is 2 or 3; each R₄ and R₅ can be the same or different;

with the proviso that either R₁ or R₂ can be taken with R₃; R₄ can be taken with R₅; and R₆ can be taken with R₇; respectively and independently, to form a substituted or unsubstituted pyrrolidine ring;

X and Y are either 1) each a hydrogen atom or
2) taken together to represent a carbonyl group;

and a pharmaceutically acceptable salt, solvate
or hydrate thereof.

5 The terms used in the above Formula I having
the following meanings when used in conjunction with
Formula I and when used in described subsequent Formulas:

C₁ to C₁₀ cycloalkyl - unsubstituted or
substituted mono- or bicyclic saturated rings such as
10 cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl,
cycloheptyl, or adamantyl or rings wherein the
substituents are one or more hydroxy, halo, amino,
protected amino, carboxy, protected carboxy, amido,
nitro, trifluoromethyl, phenyl, heterocyclic rings, C₁ to
15 C₁ acyl, C₁ to C₃ alkoxy, or C₁ to C₃ alkyl groups;

C₁ to C₆ heterocycle - unsubstituted or
substituted mono- or bicyclic rings containing 3 to 6
carbons and from one to three nitrogen, oxygen, sulfur
atoms such as azetidine, pyrrolidine, pyrazolidine,
20 piperidine, piperazine, perhydroazepine or tropane,
oxazole, thiazole, pyrazole, thiophenyl and pyranyl rings
wherein the substituents are one or more hydroxy,
protected hydroxy, halo, amino, protected amino,
monosubstituted amino, disubstituted amino, carboxy,
25 protected carboxy, amido, nitro, trifluoromethyl,
phenyl, or C₁ to C₃ alkyl groups;

C₁ to C₁₂ Alkyl - straight-chain or branched carbon chain optionally containing a C₃ to C₆ saturated or partially saturated ring, wherein the carbon chain may also optionally be partially unsaturated, such as methyl,
5 ethyl, tert-butyl, iso-propyl, 6-(cyclohexyl)-n-hexyl, allyl, n-octyl, 3-(cyclopentyl)-n-pentyl, methylcyclopropyl, and the like;

The term C₁ to C₁₂ Substituent Group indicates a group of the formula

10

-CH₂-W

wherein W is chosen from the group consisting of a hydrogen atom, C₁ to C₁₂ alkyl, C₃ to C₁₀ cycloalkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, as those
15 terms are defined herein;

The term "C₁ to C₁₂, substituted alkyl," denotes the above C₁ to C₁₂ alkyl groups that are substituted by one to three halogen, hydroxy, protected hydroxy, amino, protected amino, guanidino, C₁ to C₆ acyloxy, C₁ to C₆ acyl, nitro, carboxy, protected carboxy, carboxamide, carbonyl, carboxyl, cyano, methylsulfonylamino or C₁ to C₄ alkoxy groups. The substituted alkyl groups may be substituted once or twice with the same or with different substituents;

25

Examples of the above substituted alkyl groups include the cyanomethyl, nitromethyl, hydroxymethyl,

trityloxymethyl, propionyloxymethyl, aminomethyl, carboxymethyl, allyloxycarbonylmethyl, allyloxycarbonylaminomethyl, carbamoylmethyl, methoxymethyl, ethoxymethyl, t-butoxymethyl,

5 acetoxymethyl, chloromethyl, bromomethyl, iodomethyl, 6-hydroxyhexyl, 2,4-dichloro(n-butyl), 2-amino(iso-propyl), 2-carbamoylethyl and the like. A preferred group of examples within the above "C₁ to C₆ substituted alkyl" group includes the substituted methyl group, in other words, a methyl group substituted by the same substituents as the "C₁ to C₆ substituted alkyl" group. Examples of the substituted methyl group include groups such as protected hydroxymethyl, (e.g., tetrahydropyranyloxymethyl), acetoxymethyl,

10 carbamoylmethyl, chloromethyl, bromomethyl and iodomethyl.

15 carbamoylmethyl, chloromethyl, bromomethyl and iodomethyl.

The term "substituted phenyl" specifies a phenyl group substituted with one or more, and preferably one or two, moieties chosen from the groups consisting of

20 halogen, hydroxy, protected hydroxy, cyano, nitro, trifluoromethyl, C₁ to C₆ alkyl, C₁ to C₆ alkoxy, C₁ to C₆ acyl, C₁ to C₆ acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino,

25 (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino, carboxamide, protected carboxamide, N-(C₁ to C₆ alkyl)carboxamide, protected N-(C₁ to C₆ alkyl)carboxamide, N,N-di(C₁ to C₆ alkyl)carboxamide, trifluoromethyl, N-((C₁ to C₆ alkyl)sulfonyl)amino, N-

30 (phenylsulfonyl)amino or phenyl, substituted or

unsubstituted, such that, for example, a biphenyl or naphthyl group results.

Examples of the term "substituted phenyl" includes a mono- or di(halo)phenyl group such as 2, 3 or 5 4-chlorophenyl, 2,6-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 2, 3 or 4-bromophenyl, 3,4-dibromophenyl, 3-chloro-4-fluorophenyl, 2, 3 or 4-fluorophenyl and the like; a mono or di(hydroxy)phenyl group such as 2, 3 or 4-hydroxyphenyl, 2,4-dihydroxyphenyl, the protected-hydroxy derivatives thereof and the like; a nitrophenyl group such as 2, 3 or 4-nitrophenyl; a cyanophenyl group, for example, 2, 3 or 4-cyanophenyl; a mono- or di(alkyl)phenyl group such as 2, 3 or 4-methylphenyl, 2,4-dimethylphenyl, 2, 3 or 4-(iso-propyl)phenyl, 2, 3 or 4-ethylphenyl, 2, 3 or 4-(n-propyl)phenyl and the like; a mono or di(alkoxy)phenyl group, for example, 2,6-dimethoxyphenyl, 2, 3 or 4-methoxyphenyl, 2, 3 or 4-ethoxyphenyl, 2, 3 or 4-(isopropoxy)phenyl, 2, 3 or 4-(t-butoxy)phenyl, 3-ethoxy-20 4-methoxyphenyl and the like; 2, 3 or 4-trifluoromethylphenyl; a mono- or dicarboxyphenyl or (protected carboxy)phenyl group such as 2, 3 or 4-carboxyphenyl or 2,4-di(protected carboxy)phenyl; a mono- or di(hydroxymethyl)phenyl or (protected hydroxymethyl)phenyl such as 2, 3, or 4-(protected hydroxymethyl)phenyl or 3,4-di(hydroxymethyl)phenyl; a mono- or di(aminomethyl)phenyl or (protected aminomethyl)phenyl such as 2, 3 or 4-(aminomethyl)phenyl or 2,4-(protected aminomethyl)phenyl; or a mono- or di(N-30 (methylsulfonylamino))phenyl such as 2, 3 or 4-(N-

(methylsulfonylamino))phenyl. Also, the term "substituted phenyl" represents disubstituted phenyl groups wherein the substituents are different, for example, 3-methyl-4-hydroxyphenyl, 3-chloro-4-
5 hydroxyphenyl, 2-methoxy-4-bromophenyl, 4-ethyl-2-hydroxyphenyl, 3-hydroxy-4-nitrophenyl, 2-hydroxy 4-chlorophenyl and the like.

The terms "halo" and "halogen" refer to the fluoro, chloro, bromo or iodo groups. There can be one
10 or more halogen, which are the same or different. Preferred halogens are chloro and fluoro.

The term "C₁ to C₁₆ alkylaryl" denotes a C₁ to C₆ alkyl group substituted at any position by a phenyl or naphthyl ring. Examples of such a group include benzyl,
15 2-phenylethyl, 3-phenyl(n-propyl), 4-phenylhexyl, 3-phenyl(n-amyl), 3-phenyl(sec-butyl) and the like. Preferred C₁ to C₁₆ phenylalkyl groups are the benzyl phenylethyl napth-1-ylmethyl and napth-2-ylmethyl groups.

The term "C₁ to C₁₆ substituted alkylaryl"
20 denotes a C₁ to C₁₆ alkylaryl group substituted on the C₁ to C₆ alkyl portion with one or more, and preferably one or two, groups chosen from halogen, hydroxy, protected hydroxy, oxo, protected oxo, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino,
25 (disubstituted)amino, guanidino, heterocyclic ring, substituted heterocyclic ring, C₁ to C₆ alkoxy, C₁ to C₆ acyl, C₁ to C₆ acyloxy, nitro, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C₁ to C₆

alkyl)carboxamide, protected N-C₁ to C₆ alkyl)carboxamide, N, N-(C₁ to C₆ dialkyl)carboxamide, cyano, N-((C₁ to C₆ alkylsulfonyl)amino, thiol, C₁ to C₄ alkylthio, C₁ to C₄ alkylsulfonyl groups; and/or the phenyl group may be
5 substituted with one or more, and preferably one or two, substituents chosen from halogen, hydroxy, protected hydroxy, cyano, nitro, C₁ to C₆ alkyl, C₁ to C₆ alkoxy, C₁ to C₆ acyl, C₁ to C₆ acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl,
10 protected hydroxymethyl, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino, carboxamide, protected carboxamide, N-(C₁ to C₆ alkyl) carboxamide, protected N-(C₁ to C₆ alkyl) carboxamide, N, N-di(C₁ to C₆ alkyl)carboxamide,
15 trifluoromethyl, N-((C₁ to C₆ alkyl)sulfonyl)amino, N-(phenylsulfonyl)amino or a phenyl group, substituted or unsubstituted, for a resulting biphenyl or naphthyl group. The substituted alkyl or phenyl groups may be substituted with one or more, and preferably one or two,
20 substituents which can be the same or different.

Examples of the term "C₁ to C₁₆ substituted alkylaryl" include groups such as 2-phenyl-1-chloroethyl, 2-(4-methoxyphenyl)ethyl, 4-(2,6-dihydroxy phenyl)n-hexyl, 2-(5-cyano-3-methoxyphenyl)n-pentyl, 3-(2,6-25 dimethylphenyl)n-propyl, 4-chloro-3-aminobenzyl, 6-(4-methoxyphenyl)-3-carboxy(n-hexyl), 5-(4-aminomethylphenyl)-3-(aminomethyl)n-pentyl, 5-phenyl-3-oxo-n-pent-1-yl, (4-hydroxynaph-2-yl)methyl and the like.

The term "**(monosubstituted)amino**" refers to an amino group with one substituent chosen from the group consisting of phenyl, substituted phenyl, C₁ to C₆ alkyl, C₁ to C₆ substituted alkyl, C₁ to C₆ acyl, C₂ to C₆ alkenyl, 5 C₂ to C₆ substituted alkenyl, C₂ to C₆ alkynyl, C₂ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl and heterocyclic ring. The (monosubstituted)amino can additionally have an amino-protecting group as encompassed by the term "protected 10 (monosubstituted)amino."

The term "**(disubstituted)amino**" refers to amino groups with two substituents chosen from the group consisting of phenyl, substituted phenyl, C₁ to C₆ alkyl, C₁ to C₆ substituted alkyl, C₁ to C₆ acyl, C₂ to C₆ alkenyl, 15 C₂ to C₆ alkynyl, C₂ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl and heterocyclic ring. The two substituents can be the same or different.

The term "**pharmaceutically-acceptable salt**" encompasses those salts that form with the carboxylate anions and includes salts formed with the organic and inorganic cations such as those chosen from the alkali and alkaline earth metals, (for example, lithium, sodium, potassium, magnesium, barium and calcium); ammonium; and the organic cations (for example, dibenzylammonium, 20 benzylammonium, 2-hydroxyethylammonium, bis(2-hydroxyethyl)ammonium, phenylethylbenzylammonium, dibenzylethylenediammonium, and like cations). Other cations encompassed by the above term include the protonated form of procaine, quinine and 25

N-methylglucosamine, and the protonated forms of basic amino acids such as glycine, ornithine, histidine, phenylglycine, lysine, and arginine, and acetic acid-like counter-ions such as acetate and trifluoroacetate.

- 5 Furthermore, any zwitterionic form of the instant compounds formed by a carboxylic acid and an amino group is referred to by this term. A preferred cation for the carboxylate anion is the sodium cation. Furthermore, the term includes salts that form by standard acid-base
10 reactions with basic groups (such as amino groups) and organic or inorganic acids. Such acids include hydrochloric, sulfuric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, D-glutamic, D-camphoric, glutaric,
15 phthalic, tartaric, lauric, stearic, salicyclic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic, and the like acids.

The compounds of Formula I may also exist as solvates and hydrates. Thus, these compounds may
20 crystallize with, for example, waters of hydration, or one, a number of, or any fraction thereof of molecules of the mother liquor solvent. The solvates and hydrates of such compounds are included within the scope of this invention.

25 The term "carboxy-protecting group" as used herein refers to one of the ester derivatives of the carboxylic acid group commonly employed to block or protect the carboxylic acid group while reactions are carried out on other functional groups on the compound.

Examples of such carboxylic acid protecting groups include t-butyl, 4-nitrobenzyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl, 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl, 2,4,6-trimethylbenzyl, 5 pentamethylbenzyl, 3,4-methylenedioxybenzyl, benzhydryl, 4,4'-dimethoxytrityl, 4,4',4"-trimethoxytrityl, 2-phenylpropyl, trimethylsilyl, t-butyldimethylsilyl, phenacyl, 2,2,2-trichloroethyl, β -(trimethylsilyl)ethyl, β -(di(n-butyl)methylsilyl)ethyl, p-toluenesulfonylethyl, 10 4-nitrobenzylsulfonylethyl, allyl, cinnamyl, 1-(trimethylsilylmethyl)-propenyl and like moieties. The species of carboxy-protecting group employed is not critical so long as the derivatized carboxylic acid is stable to the conditions of subsequent reaction(s) and 15 can be removed at the appropriate point without disrupting the remainder of the molecule. Further examples of these groups are found in C.B. Reese and E. Haslam, "Protective Groups in Organic Chemistry," J.G.W. McOmie, Ed., Plenum Press, New York, NY, 1973, Chapter 5, 20 respectively, and T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 2nd ed., John Wiley and Sons, New York, NY, 1991, Chapter 5, each of which is incorporated herein by reference. A related term is "protected carboxy," which refers to a carboxy 25 group substituted with one of the above carboxy-protecting groups.

The term "hydroxy-protecting group" refers to readily cleavable groups bonded to hydroxyl groups, such as the tetrahydropyranyl, 2-methoxyprop-2-yl, 30 1-ethoxyeth-1-yl, methoxymethyl, β -methoxyethoxymethyl,

methylthiomethyl, t-butyl, t-amyl, trityl,
4-methoxytrityl, 4,4'-dimethoxytrityl,
4,4',4"-trimethoxytrityl, benzyl, allyl, trimethylsilyl,
(t-butyl)dimethylsilyl, 2,2,2-trichloroethoxycarbonyl
5 groups and the like.

Further examples of hydroxy-protecting groups are described by C.B. Reese and E. Haslam, "Protective Groups in Organic Chemistry," J.G.W. McOmie, Ed., Plenum Press, New York, NY, 1973, Chapters 3 and 4, 10 respectively, and T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," Second Edition, John Wiley and Sons, New York, NY, 1991, Chapters 2 and 3. A preferred hydroxy-protecting group is the tert-butyl group. The related term "protected hydroxy" 15 denotes a hydroxy group bonded to one of the above hydroxy protecting groups.

The term "amino-protecting group" as used herein refers to substituents of the amino group commonly 20 employed to block or protect the amino functionality while reacting other functional groups of the molecule. The term "protected (monosubstituted) amino" means there is an amino-protecting group on the monosubstituted amino nitrogen atom. In addition, the term "protected 25 carboxamide" means there is an amino-protecting group on the carboxamide nitrogen.

Examples of such amino-protecting groups include the formyl ("For") group, the trityl group, the phthalimido group, the trichloroacetyl group, the

trifluoro-acetyl group, the chloroacetyl, bromoacetyl, and iodoacetyl groups, urethane-type blocking groups, such as t-butoxycarbonyl ("Boc"), 2-(4-biphenylyl)propyl-2-oxycarbonyl ("Bpoc"), 2-phenylpropyl-2-oxycarbonyl ("Poc"), 2-(4-xenyl)isopropoxycarbonyl, 1,1-diphenylethyl-1-oxycarbonyl, 1,1-diphenylpropyl-1-oxycarbonyl, 2-(3,5-dimethoxyphenyl)propyl-2-oxycarbonyl ("Ddz"), 2-(p-toluyl)propyl-2-oxycarbonyl, cyclopentanyloxycarbonyl,

10 1-methylcyclopentanyloxycarbonyl, cyclohexanyloxy-carbonyl, 1-methylcyclohexanyloxycarbonyl, 2-methylcyclohexanyloxycarbonyl, 2-(4-toluylsulfonyl)ethoxycarbonyl, 2-(methylsulfonyl)ethoxycarbonyl, 2-(triphenylphosphino)-

15 ethoxycarbonyl, 9-fluorenylmethoxycarbonyl ("Fmoc"), 2-(trimethylsilyl)ethoxycarbonyl, allyloxycarbonyl, 1-(trimethylsilylmethyl)prop-1-enyloxycarbonyl, 5-benzisoxalylmethoxycarbonyl, 4-acetoxybenzyloxycarbonyl,

20 2,2,2-trichloroethoxycarbonyl, 2-ethynyl-2-propoxycarbonyl, cyclopropylmethoxycarbonyl, isobornyloxycarbonyl, 1-piperidyloxycarbonyl, benzyloxycarbonyl ("Cbz"), 4-phenylbenzyloxycarbonyl, 2-methylbenzyloxy-carbonyl, α-2,4,5,-

25 tetramethylbenzyloxycarbonyl ("Tmz"), 4-methoxybenzyloxycarbonyl, 4-fluorobenzyloxycarbonyl, 4-chlorobenzyloxycarbonyl, 3-chlorobenzyloxycarbonyl, 2-chlorobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, 4-bromobenzyloxycarbonyl, 3-bromobenzyloxycarbonyl,

30 4-nitrobenzyloxycarbonyl, 4-cyanobenzyloxycarbonyl,

4-(decyloxy)benzyloxycarbonyl and the like; the benzoylmethylsulfonyl group, 2,2,5,7,8-pentamethylchroman-6-sulfonyl group ("PMC") dithiasuccinoyl ("Dts"), the 2-(nitro)phenylsulfonyl group ("Nps"), the diphenyl-phosphine oxide group and like amino-protecting groups. The species of amino-protecting group employed is not critical so long as the derivatized amino group is stable to the conditions of the subsequent reaction(s) and can be removed at the appropriate point without disrupting the remainder of the compounds. Preferred amino-protecting groups are Boc, Cbz and Fmoc. Further examples of amino-protecting groups embraced by the above term are well known in organic synthesis and the peptide art and are described by, for example, T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis," 2nd ed., John Wiley and Sons, New York, NY, 1991, Chapter 7, M. Bodanzsky, "Principles of Peptide Synthesis," 1st and 2nd revised ed., Springer-Verlag, New York, NY, 1984 and 1993, and J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," 2nd ed., Pierce Chemical Co., Rockford, IL, 1984, E. Atherton and R.C. Shephard, "Solid Phase Peptide Synthesis - A Practical Approach" IRL Press, Oxford, England (1989), each of which is incorporated herein by reference. The related term "protected amino" defines an amino group substituted with an amino-protecting group discussed above.

The term "heterocycle" denotes optionally substituted five-membered or six-membered rings that have 30 1 to 4 heteroatoms, such as oxygen, sulfur and/or

nitrogen, in particular nitrogen, either alone or in conjunction with sulfur or oxygen ring atoms. These five-membered or six-membered rings may be saturated, fully unsaturated or partially unsaturated, with fully 5 saturated rings being preferred. An "amino-substituted heterocyclic ring" means any one of the above-described heterocyclic rings is substituted with at least one amino group. Preferred heterocyclic rings include morpholino, piperidinyl, piperazinyl, tetrahydrofuran, pyrrolo, and 10 tetrahydrothiophenyl.

Furthermore, the above optionally substituted five-membered or six-membered rings can optionally be fused to an aromatic 5-membered or 6-membered ring system, such as a pyridine or a triazole system, and 15 preferably to a benzene ring.

The following ring systems are examples of the heterocyclic (whether substituted or unsubstituted) radicals denoted by the term "heterocyclic ring": thienyl, furyl, pyrrolyl, imidazolyl, pyrazolyl, thiazolyl, 20 isothiazolyl, oxazolyl, isoxazolyl, triazolyl, thiadiazolyl, oxadiazolyl, tetrazolyl, thiatriazolyl, oxatriazolyl, pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, thiazinyl, oxazinyl, triazinyl, thiadiazinyl, oxadiazinyl, dithiazinyl, dioxazinyl, oxathiazinyl, 25 tetrazinyl, thiatriazinyl, oxatriazinyl, dithiadiazinyl, imidazolinyl, dihydropyrimidyl, tetrahydropyrimidyl, tetrazolo[1,5-b]pyridazinyl and purinyl, as well as benzo-fused derivatives, for example benzoxazolyl, benzthiazolyl, benzimidazolyl and indolyl.

Further specific examples of the above heterocyclic ring systems are 6-membered ring systems containing one to three nitrogen atoms. Such examples include pyridyl, such as pyrid-2-yl, pyrid-3-yl and 5 pyrid-4-yl; pyrimidyl, preferably pyrimid-2-yl and pyrimid-4-yl; triazinyl, preferably 1,3,4-triazin-2-yl and 1,3,5-triazin-4-yl; pyridazinyl, in particular pyridazin-3-yl, and pyrazinyl. The pyridine N-oxides and 10 pyridazine N-oxides, and the pyridyl, pyrimid-2-yl, pyrimid-4-yl, pyridazinyl and the 1,3,4-triazin-2-yl radicals, are a preferred group.

The substituents for the optionally substituted heterocyclic ring systems, and further examples of the 5- and 6-membered ring systems discussed above, are found 15 in W. Durckheimer et al., U.S. Pat. No. 4,278,793, issued July 14, 1981, columns 9 through 21 and columns 188 through 233, herein incorporated by reference. (In columns 33 through 188, examples of the term "heterocyclic ring" are included in the heterocyclic 20 thiomethyl groups listed under heading "A".)

The term "C₆ to C₁₅ alkyl heterocycle" denotes a C₁ to C₆ alkyl group substituted at any position by a heterocycle ring (heterocycle) from as described above, said heterocycle containing up to 14 carbon atoms, as 25 long as sum of the carbon atoms of the alkyl chain (up to 6) and the carbon atoms of the heterocycle do not exceed 15. Similarly, the term "substituted C₆ to C₁₅ alkyl heterocycle" refer to a C₆ to C₁₅ alkyl heterocycle group substituted on the alkyl portion with the same

substituents as listed for the C₁ to C₁₆ substituted alkylaryl groups and on the heterocycle as defined above for substituted heterocycle.

The above single compound or library of an
5 approximately equal molar mixture of two or more compounds has several preferred embodiments. Specifically, in the embodiment where a single compound is indicated, a preferred group of single compounds are the interior amido compounds, that is, wherein X and Y
10 are taken together to form a carbonyl moiety. A preferred group of interior amido single compounds are the dimers, thus, wherein B, AA, BB and CC are zero, except that AA can be zero or one when R₁ is a hydrogen atom and that CC can be zero or one when R₁ is a hydrogen
15 atom. In turn, a preferred group of interior amido dimers are those that are cleaved from the solid support and are not quaternized, thus, wherein R₁ is a hydrogen atom and R₁₀ is absent. A preferred group of cleaved interior amido dimer single compounds are those wherein R₁,
20 and R₂ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-
25 (naph-2-ylmethylamino)-n-butyl], S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(methylsulfinyl)eth-1-yl, S- or R-acetamido, S- or R-(N,N-dimethyl)acetamido, S- or R-(N,N-diethyl)acetamido,
S- or R-(N,N-diallyl)acetamido, S- or R-(N-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or
30

- R-(N-benzyl)acetamido, S- or R-(N,N-di(naphth-2-ylmethyl))acetamido, S- or R-(N-(naphth-2-ylmethyl))acetamido, S- or R-propionamido, S- or R-(N,N-dimethyl)propionamido, S- or R-(N,N-diethyl)propionamido,
- 5 S- or R-(N,N-diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, S- or R-(N,N-di(naphth-2-ylmethyl))propionamido, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N,N-diallyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-triallyl)-3-guanidino-n-propyl], S- or R-
- 10 [(N,N,N'-trimethyl)-3-(guanidino)-n-propyl], S- or R-[(N,N,N'-triethyl)-3-(guanidino)-n-propyl], S- or R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[3-(carboxy)-n-propyl], S- or R-[iso-propyl], S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(methoxy)benzyl, S- or R-(4-(ethoxy)benzyl, S- or R-(4-(allyloxy)benzyl,
- 15 20 S- or R-[4-hydroxybenzyl], S- or R-(n-butyl), S- or R-(n-propyl), S- or R-[(naphth-2-yl)methyl], AA is zero or one when R₁ is a hydrogen atom, CC is zero or one when R₁ is hydrogen atom, S- or R-[cyclohexylmethyl], S- or R-thiomethyl, or when either R₁ or R₂ are taken together
- 25 with R₃ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

An especially preferred group of single compounds referred to as cleaved interior amido dimers, hereafter referred to as the "Type I" amido dimers, is 30 wherein R₆ and R₈ are independently methyl, ethyl, allyl,

- benzyl, or naph-2-ylmethyl. A preferred group of "Type I" amido dimers are the "Type II" amido dimers, thus, wherein either R₁ or R₂ are each a hydrogen atom, or one of R₁ or R₂ is a hydrogen atom and the other is taken 5 together with R₃ to form an S-pyrrolidine ring. A preferred group of the Type II interior amido dimers is the N-terminal monomer as a proline residue, thus, wherein one of R₁ or R₂ is a hydrogen atom and the other is taken together with R₃ to form an S-pyrrolidine ring.
- 10 A preferred group of N-terminal proline Type II interior amido dimers occurs when R₆ is napth-2-ylmethyl and R₈ is benzyl, and more so when R₇ is S- or R-methyl, a hydrogen atom, S- or R-[3-(guanidino)-n-propyl], S- or R-[4-(N-benzylamino)-n-butyl], S-[iso-propyl], S-[2-
- 15 (methylsulfinyl)ethyl], S- or R-(n-propyl), S- or R-(hydroxymethyl), S- or R-[n-butyl], R-[(napth-2-yl)methyl], or S-phenyl, and especially so when the C-terminal residue is S- or R-alanine, thus, R₇ is a S- or R-methyl.
- 20 Another preferred group of Type II interior amido dimers has the N-terminal residue as a S-phenylalanine, and more so wherein R₆ is ethyl and R₈ is (napth-2-yl)methyl, and especially so when R₇ is S-methyl, S-(2-(methylsulfinyl)ethyl), a hydrogen atom, S-(4-(hydroxy)benzyl) or S-[(hydroxy)methyl]. Of a special 25 note within this preferred group of compounds is the compound wherein R₇ is S-methyl.
- Another preferred group of Type I interior amido dimers are wherein R₁ and R₂ are each a hydrogen atom

and R₃ is R-[{N-(naph-2-ylmethyl)indol-3-yl)methyl]. Of note within this preferred group of Type I compounds are wherein R₆ is naph-2-ylmethyl and R₈ is benzyl, and especially so when R₇ is S- or R-[3-(guanidino)-n-propyl] 5 or S- or R-[4-(benzylamino)-n-butyl].

Yet another preferred group of Type I interior amido dimers occurs wherein either R₁ or R₂ is a hydrogen atom or is taken in conjunction with R₃ to form a pyrrolidine ring, and the other is C₁ to C₁₂ acyl, C₃ to C₁₀ 10 cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle.

Another preferred group of compounds of the 15 above Formula I wherein a single compound is indicated is the interior amine compounds, in other words, wherein X and Y are each a hydrogen atom. A preferred group of these interior amine compounds is dimers, wherein B, AA, BB and CC are zero, except that AA can be zero or one 20 when R₃ is a hydrogen atom and that CC can be zero or one when R₃ is a hydrogen atom. In turn, a preferred group of interior amine dimers is those cleaved from the solid support, thus, wherein R₃ is a hydrogen atom and R₁₀ is absent. A preferred group of cleaved interior amine 25 dimers is those wherein R₆ and R₈ are independently methyl, benzyl or 4-hydroxybenzyl. A preferred group of these preferred cleaved interior dimers are referred to hereafter as "Type I cleaved interior amine dimers", that is, wherein R₃ and R₇ are independently benzyl or 4-

hydroxybenzyl. A preferred group of the Type I cleaved interior amine dimers are those wherein R₈ is benzyl, R₇ is 4-hydroxybenzyl, R₆ is methyl, and R₃ is 4-hydroxybenzyl. A more preferred group of these Type I compounds are 5 wherein a) R₁ and R₂ are the same and are methyl or a hydrogen atom; b) either R₁ or R₂ is a hydrogen atom and the other is chosen from the group consisting of methyl, isopropyl, cyclopropylmethyl, 4-hydroxymethyl, N-methylpiperidin-4-yl, and 3-(N,N-dimethylamino)-2-methyl-10 prop-2-en-1-yl. Another preferred group of Type I cleaved interior amine dimers are wherein R₈ is methyl, R₇ is benzyl, R₆ is 4-hydroxybenzyl, and R₃ is 4-hydroxybenzyl, and especially so wherein R₁ and R₂ are the same and are either a hydrogen atom or methyl, or one of 15 R₁ or R₂ is a hydrogen atom and the other is methyl.

Yet another preferred group of Type I cleaved interior amine dimers are wherein R₈ is methyl, R₇ is 4-hydroxymethyl, R₆ is benzyl, and R₃ is 4-hydroxybenzyl, and especially so wherein R₁ and R₂ are the same and are 20 either a hydrogen atom or methyl, or one of R₁ or R₂ is a hydrogen atom and the other is methyl.

Another preferred class of compounds within the invention encompassed by Formula I is a library of an approximately equimolar mixture of two or more compounds. 25 A preferred group of this library of compounds are the interior amido compounds, thus, wherein X and Y are taken together to form a carbonyl group, and especially the interior amido dimers, wherein B, AA, BB and CC are zero, except that AA can be zero or one when R₇ is a hydrogen

atom and that CC can be zero or one when R₃ is a hydrogen atom.

A preferred group of library of interior amido dimers are the resin-bound interior amido dimers, wherein 5 R₁ is a solid support and R₁₀ is absent. A preferred group of these resin bound dimers are wherein R₁ and R₂ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(t-butoxycarbonylamino)-n-butyl], S- or 10 R-[sec-butyl], S- or R-(methylsulfinyl)eth-1-yl, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N-PMC)-3-(guanidino)-n-propyl], S- or R-(t-butyloxy)methyl, S- or R-[2-(t-butyloxy)ethyl], S-phenyl, S- or R-[2-(t-butoxycarbonyl)ethyl], S- or R-[iso-propyl], S- or R-[15 (N-(t-butoxycarbonyl)indol-3-yl)methyl], S- or R-[4-hydroxybenzyl], S- or R-[4-(t-butyloxy)benzyl], S- or R-(n-propyl), S- or R-(n-butyl), S- or R-[(naphth-2-yl)methyl], S- or R-(2-carboxyethyl), S- or R-(cyclohexylmethyl), S-[(4-methoxybenzylthio)methyl], S- or 20 R-[(4-methylbenzylthio)methyl], S- or R-thiomethyl, S- or R-[4-(N-methyl-N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[4-(N-ethyl(N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[4-(N-allyl(N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[4-(N-benzyl(N-(t-butoxycarbonyl)amino)-n-butyl], 25 S- or R-[4-(N-(naphth-2-yl)(N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-acetamido, S- or R-[2-(N,N-dimethylamino)ethyl], S- or R-(N,N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or R-(N-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or R-(N- 30 benzyl)acetamido, S- or R-(N,N-di(naphth-2-

ylmethyl))acetamido, S- or R- (N-(naphth-2-ylmethyl))acetamido, S- or R- propionamido, S- or R- (N,N-dimethyl)propionamido, S- or R- (N,N-diethyl)propionamido, S- or R- (N,N-diallyl)propionamido, S- or R- (N,N-dibenzyl)propionamido, S- or R- (N,N-di(naphth-2-ylmethyl))propionamido, S- or R- [(N,N'-diallyl-N-PMC)-3-guanidino-n-propyl], S- or R- [(N,N',N''-trimethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R- [(N,N',N''-triethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R- [N,N',N'' triallyl-N-PMC-3-(guanidino)-n-propyl], S- or R- [(indol-3-yl)methyl], S- or R- [(N-(methyl)indol-3-yl)methyl], S- or R- [(N-(ethyl)indol-3-yl)methyl], S- or R- [(N-(allyl)indol-3-yl)methyl], S- or R- [(N-(benzyl)indol-3-yl)methyl], S- or R- [(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R- (4-(methoxy)benzyl, S- or R- (4-(allyloxy)benzyl, S- or R- (4-(ethoxy)benzyl, S- or R- (4-(benzoxy)benzyl, S- or R- (4-(naphth-2-ylmethoxy)benzyl, AA is one or zero when R₁ is a hydrogen atom, CC is one or zero when R₃ is a hydrogen atom, or 20 when either R₁ or R₂ are taken together with R₃ to form an S- or R- pyrrolidine or S-[4-(hydroxy)pyrrolidine].

A more preferred group of the library of resin-bound interior amido dimers, referred to hereafter as the "Type I bound amido dimers (Library)", occurs wherein R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl. A preferred group of the Type I bound amido dimers (Library) is wherein either R₁ or R₂ is a hydrogen atom or is taken in conjunction with R₃ to form a pyrrolidine ring, and the other is C₁ to C₁₂ acyl, C₃ to C₁₀ 30 cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂

substituted alkyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle.

Another preferred group of the Type I bound amido dimers (Library) is wherein either R₁ or R₂ are each a hydrogen atom, or one of R₁ or R₂ is a hydrogen atom and the other is taken together with R₃ to form an S-pyrrolidine ring.

Another preferred group of interior amido dimers (Library) are those that have been cleaved from the solid support, hereinafter referred to as "cleaved interior amido dimers (Library)", and in the above Formula I corresponds to wherein R₁ is hydrogen and R₁₀ is absent. A preferred group of cleaved interior amido dimers are wherein R₃ and R₄ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-(naphth-2-ylmethylamino)-n-butyl], S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(methylsulfinyl)eth-1-yl, S- or R-acetamido, S- or R-[2-(N,N-dimethyl)acetamido], S- or R-(N,N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or R-(N-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or R-(N-benzylacetamido, S- and R-(N-(naphth-2-ylmethyl)acetamido, S- or R-(N,N-dimethyl)propionamido, S- or R-(N,N-diethyl)propionamido,

S- or R-(N,N-diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, S- or R-(N,N-di(naphth-2-ylmethyl)propionamido, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N,N-diallyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-triallyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-trimethyl)-3-guanidino]-n-propyl], S- or R-[(N,N,N'-triethyl)-3-guanidino)-n-propyl], S- or R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[2-(carboxy)ethyl],
10 S- or R-[iso-propyl], S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-methoxy)benzyl, S- or R-(4-(ethoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-[4-hydroxybenzyl], S- or R-(n-butyl), S- or R-(n-propyl), S- or R-[(naphth-2-yl)methyl], AA is zero when R₁ is hydrogen, CC is zero or one when R₃ is a hydrogen atom, S- or R-(cyclohexylmethyl), S- or R-thiomethyl, or when either R₁ or R₂ are taken together
15 with R₃ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

A more preferred group of library of cleaved interior dimers, hereinafter referred to as "Type I
20 cleaved amido dimers", wherein R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl.
25 Specific examples of the Type I cleaved amido dimers occur when:

(1) wherein R₆ is napth-2-ylmethyl, R₃ is R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], and R₁ and R₂ are the same and are each a hydrogen atom;

5 (2) wherein R₆ is ethyl, R₃ is benzyl and R₁ and R₂ are the same and are each a hydrogen atom;

10 (3) wherein R₆ is naphth-2-ylmethyl, R₃ is S-methyl, R₁ and R₂ are the same and are each a hydrogen atom; and

(4) wherein either R₁ or R₂ is a hydrogen atom and the other is taken in conjunction with R₃ to form an S-pyrrolidine ring, and R₆ is napth-2-ylmethyl.

15 Another preferred group of compounds within the library of an approximately equimolar mixture of two or more compounds of Formula I are the interior amine compounds, thus, wherein in the above Formula I, X and Y are the same and are each a hydrogen atom. A preferred 20 library of interior amine compounds are those that are dimers, that is wherein B, AA, BB and CC are zero, except that AA can be zero or one when R₃ is a hydrogen atom and that CC can be zero or one when R₃ is a hydrogen atom. Preferred interior amine dimers (Library) are those that 25 have been cleaved from the solid support, wherein R₃ is a hydrogen atom and R₁₀ is absent. A preferred group of such cleaved interior amine dimers are wherein R₃ and R₆

are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-(naphth-2-ylmethylamino)-n-butyl], S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(2-aminoethyl), S- or R-(methylsulfinyl)eth-1-yl, S- or R-[2-(N,N-dimethylamino)ethyl], S- or R-(N,N-diethylamino)ethyl,

10 S- or R-(N,N-diallylamino)ethyl, S- or R-(N-allylamino)ethyl, S- or R-(N,N-dibenzylamino)ethyl, S- or R-(N-benzylamino)ethyl, S- and R-(N,N-di(naphth-2-ylmethylamino))ethyl, S- and R-(N-(naphth-2-ylmethylamino))ethyl, S- or R-(N-propyl)amine, S- or R-

15 (N,N-dimethylamino)propyl, S- or R-(N,N-diethylamino)propyl, S- or R-(N,N-diallylamino)propyl, S- or R-(N,N-dibenzylamino)propyl, S- or R-(N,N-di(naphth-2-ylmethylamino))propyl, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N,N-diallyl)-3-guanidino-n-propyl], S- or R-

20 [(N,N,N'-triallyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-trimethyl)-3-(guanidino)-n-propyl], S- or R-[(N,N,N'-triethyl)-3-(guanidino)-n-propyl], S- or R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[3-(hydroxy)-n-propyl], S- or R-[iso-propyl], S-

25 or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(methoxy)benzyl,

30 S- or R-(4-(ethoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-(4-hydroxybenzyl), S- or R-(n-

butyl), S- or R-(n-propyl), S- or R-[*(naphth-2-yl)methyl*], AA is zero or one when R₁ is a hydrogen atom, CC is zero or one when R₃ is a hydrogen atom, S- or R-(cyclohexylmethyl), S- or R-thiomethyl, or when either R₁ or R₂ are taken together with R₃ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine]. A preferred group of dimers within the immediately preceding preferred group occurs when R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl.

- 10 Another preferred group within the library of interior amine dimers are the resin-bound compounds, thus, wherein R₁ is a solid support and R₁₀ is absent. A preferred group of the resin-bound interior amine dimers (Library) occurs wherein R₁ and R₂ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N,N-dimethylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-methyl-N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-methyl-N-alkylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-methyl-N-benzylamino)-n-butyl], S- or R-[4-(N-(naphth-2-ylmethoxyamino)-n-butyl], S- or R-[4-(N-methyl-N-naphth-2-ylmethoxyamino)-n-butyl], S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(2-aminoethyl), S- or R-(methylsulfinyl)eth-1-yl, S- or R-acetamido, S- or R-[2-(N,N-dimethylamino)ethyl], S- or R-(N, N-diethylamino)ethyl, S- or R-(N,N-diallylamino)ethyl, S- or R-(n-allylamino)ethyl, S- or R-(N,N-dibenzylamino)ethyl, S- or R-(N-benzylamino)ethyl,

S- or R- (N,N-di(naphth-2-ylmethylamino))ethyl, S- or R- (N-(naphth-2-ylmethylamino))ethyl, S- or R- (N-propylamine),
S- or R-propionamido, S- or R- (N,N-dimethylamino)propyl,
S- or R- (N,N-diethylamino)propyl, S- or R- (N,N-
5 diallylamino)propyl, S- or R- (N,N-dibenzylamino)propyl,
S- or R- (N,N-di(naphth-2-ylmethylamino)propyl, S- or R- [3-
(N-PMC-guanidino)-n-propyl], S- or R- [(N,N'-diallyl-N-
PMC)-3-guanidino-n-propyl], S- or R- [(N,N',N'''-triallyl-
N-PMC)-3-guanidino-n-propyl], S- or R- [(N,N',N'''-
10 trimethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-
[(N,N',N'''-triethyl-N-PMC)-3-(guanidino)-n-propyl], S- or
R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl,
S- or R-[3-(hydroxy)-n-propyl], S- or R-[iso-propyl],
S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-
15 3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or
R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-
(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-
ylmethyl)indol-3-yl)methyl], S- or R-(4-(methoxy)benzyl,
S- or R-(4-(ethoxy)benzyl, S- or R-(4-(allyloxy)benzyl,
20 S- or R-[4-hydroxybenzyl], S- or R-[n-butyl], S- or R-(n-
propyl), S- or R-[(naphth-2-yl)methyl], AA is zero or one
when R₁ is a hydrogen atom, CC is zero or one when R₂ is a
hydrogen atom, S- or R-[cyclohexylmethyl], S- or R-
[thiomethyl], or when either R₁ or R₂ are taken together
25 with R₃ to form an S- or R-pyrrolidine or S-[4-
(hydroxy)pyrrolidine]. A still more preferred group
within the library of resin bound interior amine dimers
occurs when R₆ and R₈ are independently methyl, ethyl,
allyl, benzyl, or naphth-2-ylmethyl.

Another aspect of the instant invention is a method for effecting analgesia in a mammal, which comprises administering an effective amount of a single compound of Formula I in conjunction with a pharmaceutically-acceptable carrier. A preferred method of effecting analgesia in a mammal occurs when a single compound that is an interior amido dimer and further wherein B, AA, BB and CC are zero, R₁ is a hydrogen atom, R₈ is naphth-2-ylmethyl, R₃ is S-methyl, R₆ is ethyl, R₁ is S-benzyl, and R₁ and R₂ are each a hydrogen atom is used. Another preferred method of effecting analgesia in mammals utilizes a single interior amine dimer wherein further R₁ is a hydrogen atom, R₈ is benzyl, R₃ is S-methyl, R₆ is naphth-2-ylmethyl, R₁ is taken in conjunction with either R₁ or R₂ to form an S-pyrrolidine ring and the other of R₁ and R₂ a hydrogen atom.

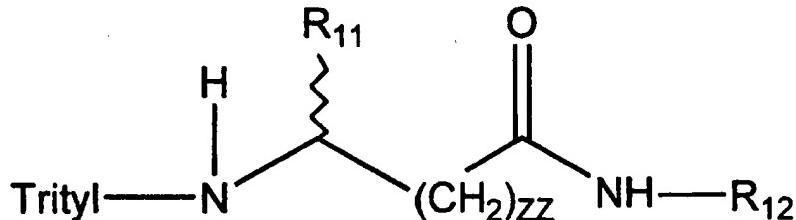
Another aspect of the instant invention is a method of effecting a decrease in the postprandial rise in blood glucose of a mammal after ingestion of a carbohydrate load by said mammal, which comprises administering an effective amount of a single compound of Formula I in conjunction with a pharmaceutically-acceptable carrier. A preferred method of affecting a decrease in the postprandial rise in the blood glucose of a mammal occurs wherein the single compound has X and Y taken together to form a carbonyl group, B, AA, BB and CC are zero, R₁ is a hydrogen atom, R₈ is benzyl, R₆ is naphth-2-ylmethyl, R₃ is R-(N-(naphth-2-ylmethyl)indol-3-ylmethyl), R₁ and R₂ are each hydrogen, R₁₀ is absent, and R₁ is chosen from the group consisting of S-(4-(N-

benzylamino)-n-butyl), R-(4-(N-benzylamino)-n-butyl), S-(3-guanidino)-n-propyl), and R-(3-guanidino)-n-propyl).

Yet another aspect of the instant invention is a method of treating microbial infections in mammals, which comprises administering an effective amount of a single compound of Formula I in conjunction with a pharmaceutically-acceptable carrier. A preferred method of treating microbial infections in mammals occurs when wherein the single compound has X and Y taken together to form a carbonyl group, B, AA, BB and CC are zero, R₅ is a hydrogen atom, R₆ is benzyl, R₇ is naphth-2-ylmethyl, R₈ is R-(N-(naphth-2-ylmethyl)indol-3-ylmethyl), R₁ and R₂ are each hydrogen, R₁₀ is absent, and R₉ is chosen from the group consisting of S-(4-(N-benzylamino)-n-butyl), R-(4-(N-benzylamino)-n-butyl), S-(3-guanidino)-n-propyl), and R-(3-guanidino)-n-propyl).

Another aspect of the instant invention is a method of step-wise N-alkylation of the amide bond of the N-terminal residue of a compound of the Formula (II):

(II)



Wherein:

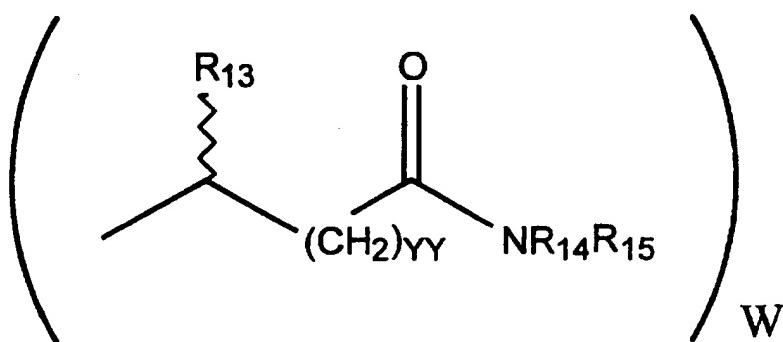
R_{11} is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocyclic, or a substituted C₆ to C₁₅ alkyl heterocycle;

ZZ is from zero to five;

And R_{12} is a solid support or a group of the Formula (III):

(III)

10



W

Wherein R_{14} is a C₁ to C₁₈ substituent group;

Wherein W is 0 to 4;

R_{13} is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl,

15

a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₁₅ is a solid support (when W is one) or a bond to the preceding methylene group (when W is from two to
5 four);

YY is from zero to five;

Wherein the compound of the above formula is
a) first reacted under anhydrous conditions in an inert atmosphere with an excess amount non-nucleophilic base
10 having a pKa between about 18 to about 40; then
b) reacting the resulting anion under anhydrous conditions in an inert atmosphere in a polar aprotic solvent with an excess amount of an alkylating agent of the formula

(LG) - Q

15 Wherein LG is leaving group;

Q is a C₁ to C₁₈ substituent groups as defined above for Formula (I);

and repeating steps a) and b) as necessary to drive the alkylation to completion;

20 with the proviso that all previous internal backbone amide bonds have been previously alkylated with a C₁ to C₁₈ substituent group and, when W is from 2 to 4,

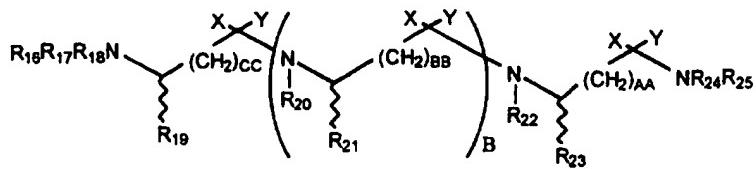
all of the R₁₄ groups are not the same C₁ to C₁₈ substituent group.

A preferred method of step-wise N-alkylation occurs when LG is iodo or bromo and the -CH₂-Q moiety is 5 methyl, ethyl, allyl, benzyl or naphth-2-ylmethyl. A further preferred method of step-wise N-alkylation occurs wherein R₁₁ and R₁₃ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(t- 10 butoxycarbonylamino)-n-butyl], S- or R-[4-(amino)-n- butyl], S- or R-[sec-butyl], S- or R-(methylsulfinyl)eth- 1-yl, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N-PMC)-3-(guanidino)-n-propyl], S- or R-(t-butoxy)methyl, S- or R-[2-(t-butoxy)ethyl], S-phenyl, S- or R-(3-(2- 15 butoxycarbonyl)ethyl), S- or R-[iso-propyl], S- or R-[(N-(t-butoxycarbonyl)indol-3-yl)methyl], S- or R-[4-hydroxybenzyl], S- or R-[(4-(t-butoxy))benzyl], S- or R-[n-propyl], S- or R-(n-butyl), S- or R-[(naphth-2-yl)methyl], S- or R-(cyclohexylmethyl), S-[(4- 20 methoxybenzylthio)methyl], S-[(4-methylbenzylthio)methyl], S- or R-thiomethyl, S- or R-[4-(N-methyl-(N-(t-butoxycarbonyl))amino)-n-butyl], S- or R-[4-(N-ethyl-(N-(t-butoxycarbonyl))amino)-n-butyl], S- or R-[4-(N-allyl-(N-(t-butoxycarbonyl))amino)-n-butyl], S- 25 or R-[4-(N-benzyl-(N-(t-butoxycarbonyl))amino)-n-butyl], S- or R-[4-(N-(naphth-2-yl)-(N-(t-butoxycarbonyl))amino)-n-butyl], S- or R-[2-(N,N-dimethyl)acetamido], S- or R-acetamido, S- or R-(N, N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or R-(n-allyl)acetamido, S- or 30 R-(N,N-dibenzyl)acetamido, S- or R-(N-benzyl)acetamido,

S- or R-(N,N-di(naphth-2-ylmethyl))acetamido, S- or R-(N-(naphth-2-ylmethyl))acetamido, S- or R-n-propylamine, S- or R-propionamido, S- or R-(N,N-dimethyl)propionamido, S- or R-(N,N-diethyl)propionamido, S- or R-(N,N-
5 diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, S- or R-(N,N-di(naphth-2-ylmethyl)propionamido, S- or R-[
[(N,N'-diallyl-N-PMC)-3-guanidino-n-propyl], S- or R-[
[(N,N',N''-trimethyl-N-PMC)-3-(guanidino)-n-propyl], S-
or R-[
[(N,N',N''-triethyl-N-PMC)-3-(guanidino)-n-propyl], S-
10 S- or R-[N,N',N''-triallyl-N-PMC)-3-guanidino-n-propyl],
S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-
3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S-
or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-
benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-
15 ylmethyl)indol-3-yl)methyl], S- or R-(4-(methoxy)benzyl,
S- or R-(4-(ethoxy)benzyl, S- or R-(4-(allyloxy)benzyl,
S- or R-(4-(benzoxy)benzyl, S- or R-(4-(naphth-2-
ylnethoxy)benzyl, ZZ is one or zero when R₁₁ is a hydrogen
atom, YY is one or zero when R₁₃ is a hydrogen atom, or
20 when either R₁₃ is taken together with R₁₄ to form an S- or
R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

Another aspect of the instant invention utilizes the positional scanning method and is a method of synthesizing and testing for biological activity a
25 library of an approximately equimolar amount of compounds of the following Formula (IV):

(IV)



Wherein in the above Formula (IV) :

R₁₉, R₂₁ and R₂₃ independently are a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₂₅ is a hydrogen atom or a solid support;

R₂₀, R₂₂ and R₂₄ are independently a C₁ to C₁₈ substituent group;

AA, BB and CC are independently 0 to 5;

B is from 0 to 3;

X and Y are taken together to form a carbonyl group or are separate and are each a hydrogen atom;

R₁₆, R₁₇ and R₁₈ independently are a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted

C₆ to C₁₅ alkyl heterocycle; R₁₆ is optionally present as a C₁ to C₁₈ substituent group when R₁ and R₂ are other than a hydrogen atom or an amino protecting group;

Wherein said library of compounds is composed
5 of SL physically separate sublibraries; wherein SL is
equal to (2B + 4);

Further wherein each sublibrary is composed of physically separate mixtures, wherein the number of said mixtures is equal to the number of different substituents
10 incorporated at R_{fix}, which R_{fix} can be any one of R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, or R₂₄ in the above Formula IV;

Wherein the compounds of the above Formula IV are synthesized and tested as follows:

(a) For each sublibrary SL, choosing R_{fix}
15 from R₁₉, R₂₀, R₂₁, R₂₂, R₂₃, or R₂₄;

(b) Dividing a solid support into approximately equal separate portions with the number of portions equal to the number of substituents to be incorporated at R₂₃, and couple each physically separate
20 portions of solid support to one of the monomers containing a single substituent at R₂₃, then mixing all of said physically separate portions;

(c) Dividing the mixed solid support from step (a) into approximately equal separate portions in a
25 number equal to the number of different substituents to

be incorporated at R_{24} by alkylation, alkylating each physically separate solid support mixtures with one alkyl group, then mixing said resins;

5 (d) When B is 1 through 3, dividing each of said solid support portions into a number of approximately equal separate portions, said number equal to the number of substituents at R_{21} , coupling one of the monomers containing a single substituent R_{23} to each separate solid support portion then mixing said portions;

10 (e) When B is 1 to 3, separating said mixture of solid support portions into a number of approximately equal separate portions, said number equal to the number of alkyl substituents at R_{20} , alkyllating each physically separate portion with one such alkyllating agent, and mixing all the resultant solid support portions;

(f) Optionally repeating steps (d) and (e) one or two times when B is two or three, respectively;

20 (g) Dividing the mixture of solid support portions from either step (c), (e), or step (f) into approximately equal separate portions equal to the number of substituents to be placed at R_{19} , coupling one such monomer containing a single R_{19} , to each physically separate solid support portion, and mixing said portions;

(h) Dividing the mixture of portions from step (g) into a number of approximately equal separate portions, said number equal to the number of alkyl substituents at R_{22} to be utilized, alkylating each said 5 separate portion with a single alkyl group R_{22} ;

(i) Optionally adding R_1 , and/or R_{18} by reductive alkylation;

(j) Optionally adding the quaternary substituent R_{16} ;

10 (k) Optionally reducing the interior amides, thus converting X and Y taken together are a carbonyl oxygen to wherein each X and Y is a hydrogen atom; and

15 (l) Cleaving said molecules from the solid support;

(m) Testing each portion of each SL sublibraries in the appropriate biological screen or screens; and determining from the results of said screens which substituent at R_{fix} is the best.

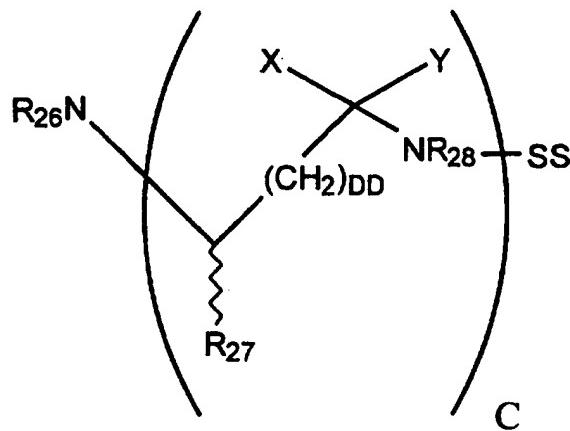
20 (n) Optionally synthesizing the molecule of Formula (I) containing the best (R_{fix}) substituent at R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , or R_{24} ;

With the proviso that for each sublibrary SL
the first solid support mixing step immediately following
the introduction of R_{fix} is omitted;

Further wherein:

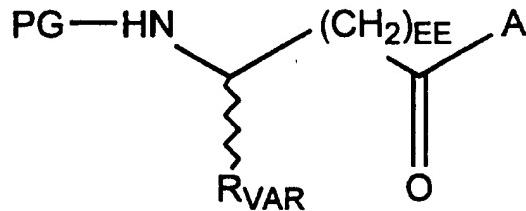
5 (1) each coupling step in the above series
of steps ((b), (d), (f) and (g)) involves a substrate of
the Formula (V):

(V)



With an excess of an active acylating form of
10 the monomer of the Formula (VI):

(VI)



Wherein in the above Formulas (V) and (VI):

SS is a solid support;

R₂₆ are two hydrogen atoms each bound to the
5 nitrogen atom;

R₂₈ is a C₁ to C₁₈ substituent group;

R₂₇ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl,
10 a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R_{VAR} can be the same or different as R₂₇, and is chosen from the same group of substituents as R₂₇;

DD and EE are independently 0 to 5;

15 X and Y are either taken together to form a carbonyl oxygen;

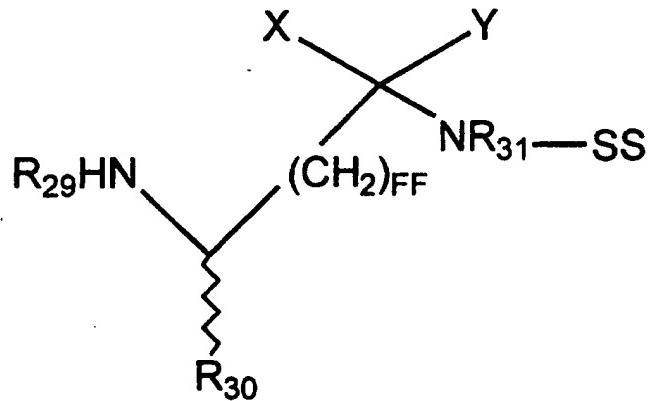
PG is an amino protecting group other than trityl;

20 A is a group, when taken with the preceding carbonyl group; that forms an active acylating agent; and

C is from 0 to 4;

(2) Each alkylation step in the above steps (c), (e), (f) and (h) requires reacting a substrate of the Formula (VII):

(VII)



With an excess of an alkylating agent of the
Formula (VIII):

5 (VIII)

(LG) - Q

Under anhydrous conditions, and an inert
atmosphere in a polar, aprotic solvent;

Wherein in the above Formulas (VII) and (VIII):

LG is a leaving group under the conditions of
10 the alkylation;

Q is a C₁ to C₁₈ substituent group as defined
above in Formula (I);

FF is 0 to 5;

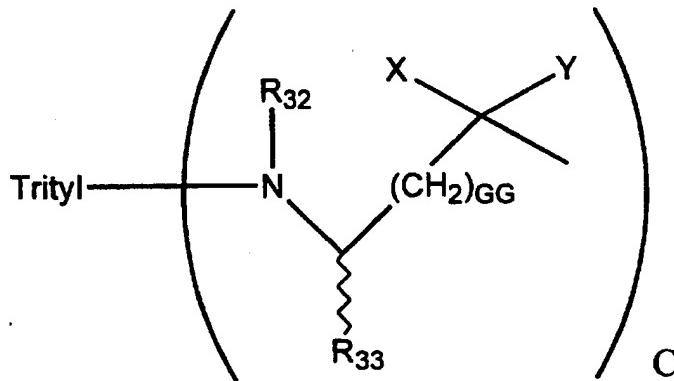
X and Y are taken together to form a carbonyl
15 oxygen;

R_{31} is a hydrogen atom when R_{29} is a trityl group or is a C_1 to C_{18} substituent group;

R_{30} is independently a hydrogen atom, C_1 to C_{12} alkyl, C_1 to C_{12} substituted alkyl, phenyl, substituted 5 phenyl, C_1 to C_{16} alkylaryl, C_1 to C_{16} substituted alkylaryl, a C_6 to C_{15} alkyl heterocycle, or a substituted C_6 to C_{15} alkyl heterocycle; and

R_{29} is a trityl group when R_{31} is a hydrogen atom or is a group of the Formula (IX):

10 (IX)



Wherein in the above Formula (IX):

X and Y are as X and Y above;

GG is 0 to 5;

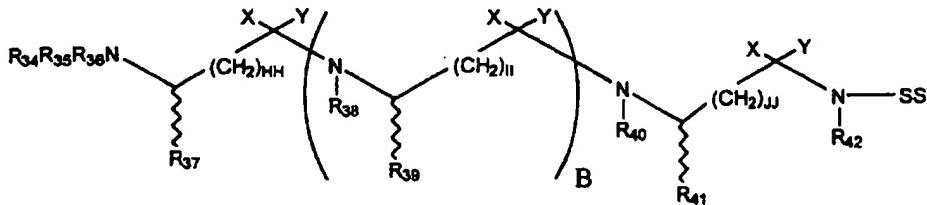
C is from 1 to 4;

15 R_{33} is independently a hydrogen atom, C_1 to C_{12} alkyl, C_1 to C_{12} substituted alkyl, phenyl, substituted phenyl, C_1 to C_{16} alkylaryl, C_1 to C_{16} substituted alkylaryl, a C_6 to C_{15} alkyl heterocycle, or a substituted C_6 to C_{15} alkyl heterocycle; and

R_{32} is a hydrogen atom if R_{32} is bonded to the N-terminal amino group or otherwise it is a C_1 to C_{18} substituent wherein one such C_1 to C_{18} substituent differs from the other substituents;

5 (3) Reductive alkylation of the N-terminal nitrogen group as described above in step (i) of a compound of the Formula (X) :

(X)



10 Under mildly acidic conditions with a ketone or aldehyde containing the R_{34} and/or R_{35} groups followed by the treatment of a reducing agent;

Wherein in the above Formula (X) :

X and Y are taken together to form a carbonyl
15 oxygen;

HH, II, and JJ are independently 0 to 5;

B is from 0 to 3;

R_{39} , R_{41} and R_{37} are the same or different and are chosen from the group consisting of independently a
20 hydrogen atom, C_1 to C_{12} alkyl, C_1 to C_{12} substituted alkyl, phenyl, substituted phenyl, C_1 to C_{16} alkylaryl, C_1 to C_{16}

substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₄₀ and R₄₂ are different and are a C₁ to C₁₈ substituent group;

5 R₃₄ or R₃₅ when B is 0, is optionally one or two hydrogen atoms attached to the nitrogen atom, or is a optionally one or more, same or different groups, chosen from the group consisting of a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

15 R₃₆ is a hydrogen or a bond to a R₃₄ or R₃₅ before the reduction occurs, and when B is from 1 to 3; R₃₈ is a C₁ to C₁₈ substituent group different from at least one other R₄₀, R₄₂ or R₃₈ groups;

20 (4) Optional reduction of the amide bonds as described above in step (j) of a compound of Formula (X) wherein X and Y are taken together to form a carbonyl groups, before or after it is cleaved from the solid support, using a boron reducing agent.

25 (5) Optionally quaternization of the terminal amino groups with an excess amount of alkylating agent of above of the formula:

(LG) -Q

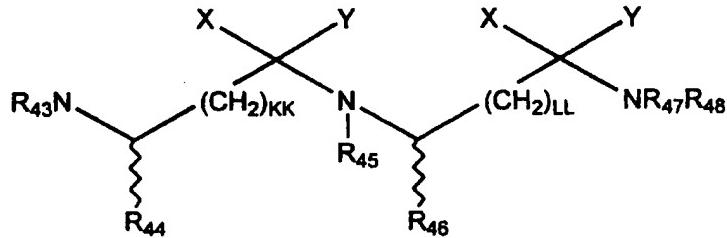
Where (LG) and Q have the same meanings as above in a polar, aprotic solvent.

It will be obvious to one skilled in the art that, in the above positional scanning method, the 5 substituents at R₃₄, R₃₅ and R₃₆ can be designated as "R_{fix}", such that the number of sublibraries SL would be increased by one, two or three, respectively, depending on how many of these three "R" groups are varied.

Yet another aspect of the invention is an 10 iterative synthetic approach wherein the method for the iterative synthesis and screening of a library of an approximately equimolar amount of compounds of the Formula (XI):

(XI)

15



Wherein in the above Formula (XI):

R₄₈ is a hydrogen atom or a solid support;

R₄₅ and R₄₇ are different and are each a C₁ to C₁₈ substituent group;

R_{44} and R_{46} are independently chosen from the group consisting of independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, 5 a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

X and Y are either taken together to form a carbonyl group or are separate and are each a hydrogen atom;

10 R_{43} is one or two hydrogen atoms, or groups of the formula R_a, R_b and R_c, wherein R_a and R_b independently are a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, 15 or a substituted C₆ to C₁₅ alkyl heterocycle; R_c is optionally present as a C₁ to C₁₈ substituent group when R_a and R_b are other than a hydrogen atom or an amino protecting group; and

20 Wherein the method comprises:

(a) Splitting a solid support into a number of approximately equal, separate portions, the number of said portions being equal to the number of monomers containing different substituent groups at R₄₆;

(b) Coupling a monomer containing one of the number of substituent groups at R₄₆, to a separate portion of the solid support;

5 (c) Mixing all of the separate portions of solid support;

(d) Splitting the solid support mixture into approximately equal, separate portions, the number of portions equal to the number of different substituents to be added at R₄₇;

10 (e) Alkylating each separate portion of solid support with a single alkylating agent, each agent containing a unique alkyl group at R₄₇, thus adding a single alkyl group at R₄₇ to the plurality of the compounds bonded to each separate portion of solid
15 support;

(f) Mixing all of the separate portions of solid support ;

20 (g) Splitting the solid support mixture into a number of approximately equal separate portions, the number of said portions equal to the number of different substituents to be added at R₄₄;

25 (h) Coupling each monomer containing a single substituent group at R₄₄ to a separate portion of the solid support, thus coupling a single different

monomer to the plurality of the compounds bonded to each separate portion of the resin;

(i) Splitting each of the separate portions of solid support into a number of approximately 5 equal physically-separate portions, wherein the number of portions is equal to the number of different substituents to be added by alkylation at R₄₅;

(j) Alkylating each separate portion of solid support with a separate alkylating agent containing 10 a single different R₄₅ group, thus adding a single different alkyl group at R₄₅ to the plurality of the compound bonded to each separate portion of the resin;

(k) Cleaving the generated compound mixtures of Formula (XI) from each separate portion of 15 solid support and testing each separate mixture from each separate portion of solid support in the appropriate biological screen or screens, and determining from the results of said screens which mixture contains the best combination of substituents at R₄₄ and R₄₅;

20 (l) Repeating steps (a) through (e), wherein the substituents at R₄₆ and R₄₇ are the same used in said original steps (a) through (e);

(m) Coupling the monomer containing the most active R₄₄ substituent to each of the separate 25 portions of resin from step (l);

(n) Alkylating each of the portions from step (m) with the best alkyl group at R₄₅ determined in step (k);

(o) Cleaving each separate mixture of 5 compounds of the above Formula (XI) from the solid support, testing each separate mixture of compounds in the same biological screens as in step (k), and determining the most active substituent at R₄, in those screens;

10 (p) Repeating steps (a) and (b), wherein the same group of monomers containing the various substituent R₄₆ are used as in the original step (a);

(q) Alkylating each separate resin portion from step (p) with an alkylating agent placing 15 the best alkyl group at R₄, as such alkyl group was determined in step (o);

(r) Coupling to each separate portion of resin the monomer containing the best R₄₄ substituent as such substituent was determined in step (k);

20 (s) Alkylating each separate portion of resin with a group that was the best alkyl group R₄₅ as such group determined in step (k);

(t) Cleaving each separate compound from the solid support, and testing each separate mixture of 25 compound separately in the same screens as in steps (o)

and (k) in order to determine the best substituents at R₄₆:

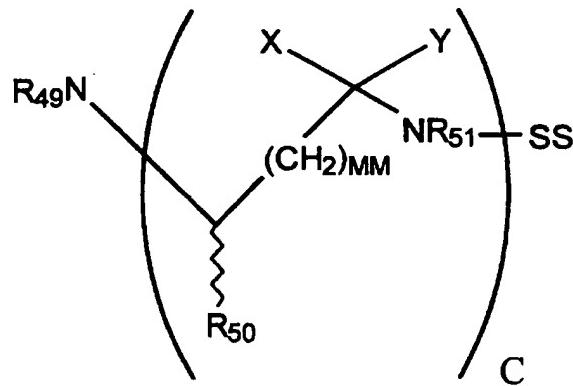
(aa) Optionally reductively alkylating and quaternizing the N-terminal amino group (R₄₃) , either 5 before or after cleavage of the compound from the solid support; and

(bb) Optionally reducing the interior amide groups before or after cleavage of the compound 10 from the solid support such that X and Y in Formula (XI) are each a hydrogen atom;

Further wherein:

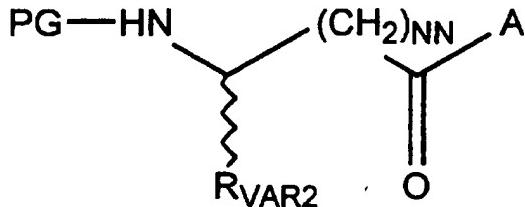
(1) each of the above coupling steps (b), (h), (l), (m), (p) or (r), involves a substrate of the Formula (XII):

15 (XII)



With an excess of an active acylating form of the monomer of the Formula (XIII):

(XIII)



5

Wherein in the above Formulas (XII) and (XIII):

SS is a solid support;

R₄₉ is two hydrogen atoms;

R₅₁ is a C₁ to C₁₈ substituent group

10 R₅₀ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

15 R_{VAR2} can be the same or different as R₅₀ and is chosen from the same group of substituents as R₅₀;

MM and NN are independently 0 to 5;

X and Y are either taken together to form a carbonyl group or are separate and are each a hydrogen atom;

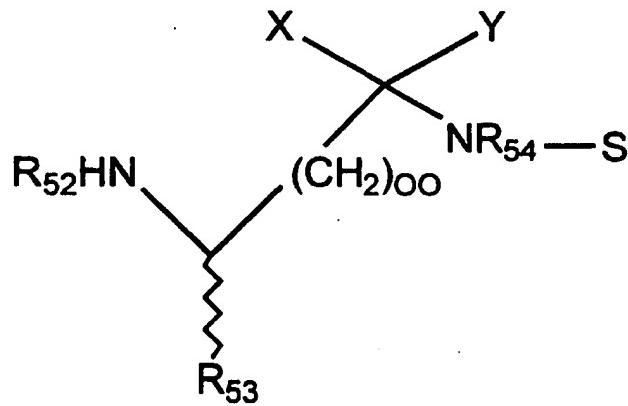
20 PG is an amino protecting group other than trityl;

A is a group, when taken with the preceding carbonyl group; that forms an active acylating agent; and

C is 0 or 1;

(2) Each of the above alkylating steps (e), (j), (l), (m), (q) and (s), requires reacting a substrate of the Formula (XIV) :

5 (XIV)



With an excess of an alkylating agent of the Formula (XV) :

(XV)

(LG) - Q

10 Under anhydrous conditions, and an inert atmosphere in a polar, aprotic solvent;

Wherein in the above Formulas (XIV) and (XV) :

LG is a leaving group under the conditions of the alkylation;

Q is a C₁ to C₁₈ substituent group;

OO is 0 to 5;

X and Y are taken together to form a carbonyl group; or are separate and are each a hydrogen atom;

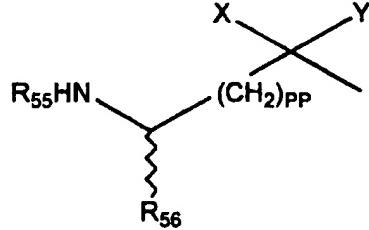
5 R₅₄ is a hydrogen atom if R₅₂ is a trityl group or is a C₁ to C₁₈ substituent group;

R₅₃ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl,
10 a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₅₂ is a trityl group if R₅₄ is a hydrogen atom or is a group of the Formula (XVI):

(XVI)

15



Wherein in the above Formula (XVI):

X and Y are as X and Y above;

PP is 0 to 5;

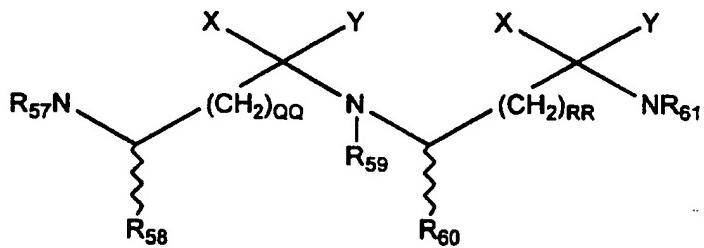
R₅₅ is a trityl group;

20 R₅₆ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl,

a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle; and

(3) Optional reductive alkylation of
5 the N-terminal nitrogen group of a compound of the
Formula (XVII) :

(XVII)



10 Under mildly acidic conditions with a ketone or aldehyde containing the R₁ and/or R₂ groups followed by treatment with a reducing agent;

Wherein in the above Formula (XVII) :

X and Y are taken together to form a carbonyl
15 group;

QQ and RR are independently 0 to 5;

R₅₈ and R₆₀ are the same or different and are chosen from the group consisting of independently of a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl,
20 phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R_{59} and R_{61} are the same or different and are a C_1 to C_{18} substituent group;

R_{57} is either two hydrogen atoms attached to the nitrogen atom, or is a single hydrogen atom and 5 another group bonded to the nitrogen atom which groups is selected from the group consisting of a hydrogen atom, an amino protecting group, C_1 to C_{12} acyl, C_3 to C_{10} cycloalkyl, C_3 to C_6 heterocycle, C_1 to C_{12} alkyl, C_1 to C_{12} substituted alkyl, C_1 to C_{16} alkylaryl, C_1 to C_{16} substituted 10 alkylaryl, a C_6 to C_{15} alkyl heterocycle, or a substituted C_6 to C_{15} alkyl heterocycle;

(4) Optional reduction of the amide bonds of a compound of Formula (XI), before or after it is cleaved from the solid support, using a boron-based 15 reducing agent, such as borane or sodium borohydride, and the like.

In the above iterative synthetic approach, it would be obvious to one skilled in the art the method could easily be extended to encompass a library of all of 20 the compounds of Formula I, in other words, such that repeating the coupling; alkylation and testing steps described above be applied to compounds within the scope of Formula I, wherein B is 1, 2, or 3. Furthermore, it would be obvious to one skilled in the art that 25 substituent at R_1 , R_2 and R_{10} would be separate variables that could be synthesized and screened by the above iterative method. Due to their location in the molecule, these substituents, if present, would be screened to find

the best substituent before the N-terminal monomer and the attendant N-alkyl group could be determined.

Finally, one skilled in the art would be able to combine the above iterative and positional scanning approaches in order to conserve resources and time. For example, in libraries where B in the above Formula I is 1, 2, or 3, the iterative approach could be used to determine the optimum substituents on the last two variable substituents and the optimum substituents at the remaining positions could be determined by the positional scanning approach.

Simultaneous multiple solid phase methodology (Merrifield, R.B., J. Am. Chem. Soc., 85:2149 (1963)) was the basic technology used to synthesize and design the peptidomimetic library set forth in Formula I. A solid phase-based synthetic method was developed to successively alkylate each amide bond following its formation. In this library, different alkylating agents were used to create increased molecular diversity and to eliminate the hydrogen bonding potential of the amide functionality. Optionally, the N-terminal nitrogen can be reductively alkylated and quaternized and the interior amide bonds can be reduced (i.e., X and Y are each a hydrogen atom). In Formula I, when B is 2 or 3, R₄ and R₅ do not have to be the same as the other R₄ and R₅ groups present in the molecule. Cleavage from the solid support led to peptidomimetics of the Formula I (wherein R₆ is hydrogen, each having diversity positions at the amino acid side chain positions (R₃, R₅ and R₇), at the

amide alkyl groups (R_4 , R_6 , R_8), and at the N-terminal groups (R_1 , R_2 and R_{10}).

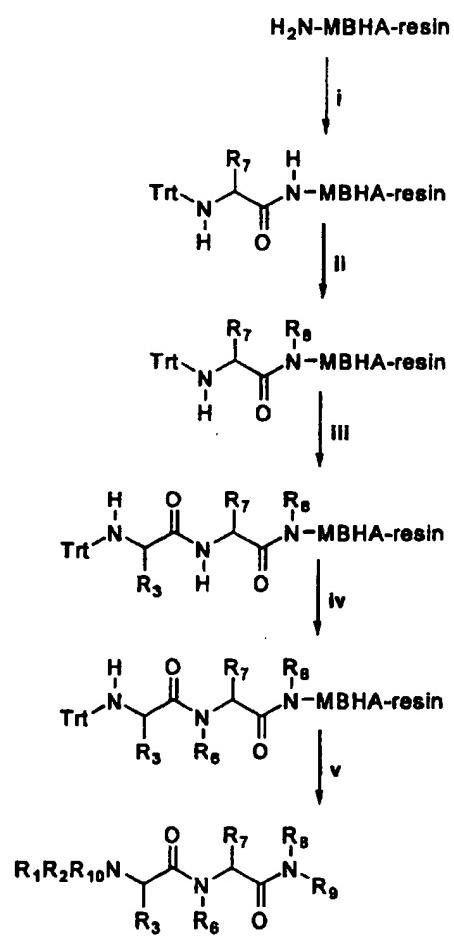
Although a number of methods for the permethylation of peptides in solution (Hakomori, S.-I., 5 J. Biochem 55:205 (1964); Vilkas, E., et al., Tetrahedron Letters, 26:3089 (1968); Challis, B.C., et al., The Chemistry of Amides, Zabicky, J. Ed.; Interscience: New York, 1970, pp. 731-857; are known, the permethylation of resin-bound peptides, using sodium hydride for the 10 formation of amide anions, was reported only recently (Ostresh et al., Proc. Natl. Acad. Sci. USA 91:11138-11142 (1994)). For the purpose of a stepwise alkylation following each amino acid coupling on the solid support, lithium t-butoxide was found to be more 15 effective for the successive formation of the amide anions.

As an important prerequisite for the synthesis of this library, reproducible conditions for the N-amide alkylations had to be established for the base treatment 20 of solid phase-bound amino acids or peptides. The reactions were carried out under an anhydrous nitrogen atmosphere, and the amino acid or peptide resin of interest was treated with excess lithium t-butoxide in tetrahydrofuran. Following removal of excess base, the 25 alkylating agent in an aprotic, polar solvent such as dimethyl sulfoxide was reacted with the resin-bound compound. The alkylation reaction mixture was then removed and the base and alkylation treatments were repeated to drive the alkylation reaction to completion.

Potential racemization during alkylation was studied using analytical reversed-phase high performance liquid chromatography (RP-HPLC); the four possible permethylated stereoisomers of Phe-Leu-NH₂ were used as reference standards. (Ostresh, J.M., et al., *Peptides 94: Proceedings of the 23rd European Peptide Symposium*, Maia, H.L.S. Ed.; Escom: Leiden, 1995, pp. 416-417). The maximum percentage of racemization found following repeated base and methylation treatments was < 1%.

10

The techniques for the synthesis of the selectively N-alkylated compounds of Formula I are well known in the art, with the exception of the selective N-alkylation procedures discussed above. These techniques can be conveniently discussed in conjunction with Scheme 1:

SCHEME 1

In the above Scheme 1, Reaction "i" represents the coupling of the C-terminus of peptide-like residue to an amino-derivatized solid support. For instance, an peptide-like residue with amino terminus protected by a 5 base or weak-acid labile protecting group, such as Fmoc, is converted to a good acylating agent *in situ* using known reagents and conditions. Such reagents include carbodiimide reagents (eg. N,N'-dicyclohexylcarbodiimide (DCC) and N,N'-diiso-propylcarbodiimide in conjunction 10 with 1-hydroxybenzotriazole) in an aprotic, polar solvent such as DMF. (Such couplings were repeated if necessary.) In the reaction labelled as "ii" in Scheme 1, the N-terminal protecting group was removed and replaced with a trityl group, which was found to protect such group 15 during the next interior, selective N-alkylation step. Under anhydrous conditions and an inert atmosphere, the tritylated residue was treated with an excess amount of lithium t-butoxide in a polar, aprotic solvent (such as tetrahydrofuran), followed by the addition of an excess 20 of an alkylating agent of the formula

(LG) -Q

wherein LG is a good leaving group under the S_n2 conditions of the reaction (such as bromo, iodo, tosyl, triflate and the like), and Q forms a C₁ to C₁₈ linear, 25 branched, cyclic, saturated, partially or fully unsaturated alkyl group, as described above in conjunction with the "C₁ to C₁₈ substituent group" of R₄, R₆, and R₈ of Formula I. Multiple repetitions of such alkylating

conditions are often necessary. The reaction denoted as "iii" in the above Scheme 1 denotes the steps necessary to couple a second (and any additional) monomer (as defined in Formula II) the recently N-alkylated resin-bound residue. Thus, the trityl protecting group is removed under weakly acidic conditions (2% trifluoroacetic acid), the deprotected molecule neutralized then coupled with and FMOC-protected amino acid (or one bearing an equivalent protecting group) using the same conditions for coupling the first residue to the amino-derivatized resin. The reaction labelled "iv" in Scheme 1 is a repeat of the selective N-alkylation procedure, including the preliminary N-protection steps, of reaction "ii". Reaction "v" shows the removal of the trityl group from the amino-terminus of the bound residue as before followed by the removal of the selectively-N-alkylated molecule from the amino-derivatized residue with hydrogen fluoride. One skilled in the art would recognize that subsequent residues could be added then selectively alkylated before removal of the amino-derivatized according to the steps set forth in Scheme 1, thus yielding compounds of Formula I wherein B is 1, 2 or 3. Furthermore, while still attached to the resin, it is advantages to derivatize the N-terminal amino group to form compounds wherein at least one of R₁ and R₂ is other than hydrogen, and where possibly R₁₀ is present. R₁ and R₂ groups are most frequently added by the process of reductive alkylation, as set forth (Borch, R.F., et al., J. Am. Chem. Soc., 93:2897 (1971); Coy, D.H., et al., Tetrahedron, 44:835 (1988); Staňková, M., et al., Drug Development Research, 33:146 (1994) (herein

incorporated by reference). Thus, appropriate aldehyde or ketone is added to the resin-bound compounds under mildly acidic conditions to effect Schiff-base (imine) formation, which imine is reduced to the substituted 5 amine by sodium cyanoborohydride, or other mild reducing agent. Additionally, the free amino terminus can be acylated with a C₁ to C₁₂ acyl group, using well-known conditions as described in Staňková, M., *et al.*, Drug Development Research, 33:146 (1994) (herein incorporated 10 by reference). It is preferable, however, to add the R₁₀ (alkyl) substituent before reductive alkylation. Such an alkylation proceeds under the same alkylation conditions used for the R₄, R₆, and R₈ groups. Finally, while still resin-bound, or after cleavage from the resin, the 15 interior amide groups can be reduced (i.e. X and Y taken together form a carbonyl group to X and Y are each a hydrogen atom.) Such a reduction is known in the art (see, for instance, Dooley, C.T., *et al.*, Analgesia, INRC Proceedings, 1:400 (1995)). Thus, for both situations 20 (i.e., when R₉ is a hydrogen atom or a solid (resin)) mild, soluble hydrogenation catalysts such as a boric acid/trimethylborate/borane-tetrahydrofuran combination can be used.

Individual model compounds were used to study 25 the modification of amino acid side chains during the alkylation conditions. Fifty N-trityl (triphenylmethyl; Trt) dipeptide resins, designated Trt-O-Leu-MBHA resin (MBHA = p-methylbenzhydrylamine) were alkylated where O represents a single proteinogenic L-amino acid, their D- 30 counterparts, or 11 other individual "unnatural" amino

acids. Aspartic acid was excluded from the 20 proteinogenic amino acids, since multiple products were formed following base treatment and alkylation. Methyl iodide, allyl bromide and benzyl bromide were used 5 initially as alkylating agents. The individual crude alkylated products were analyzed by RP-HPLC and matrix assisted laser desorption ionization-mass spectroscopy (MALDI-MS) to determine their purity and identity.

(Ostresh, J.M., et al., Peptides 94: Proceedings of the 10 23rd European Peptide Symposium, Maia, H.L.S. Ed.; Escom: Leiden, 1995, pp. 463-464). During the alkylation procedure, the functional groups of the amino acid side chains were reproducibly modified. Based on preliminary evidence the following modifications were observed: 1)

15 the ε-monoalkyl amine was formed during alkylation when the ε-amino group of lysine was protected with Boc; 2) the unprotected amide functionality of the side chains of L-asparagine and L-glutamine, when alkylated with any of the three alkylating agents, yielded dialkyl amides,

20 whereas allylation and benzylation of the D-isomers led to mono and dialkyl amides, indicating stereochemical hindrance of the diastereomers; 3) the 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) protected arginine side chain yielded the trimethyl derivative following 25 permethylation and di- and triallyl derivatives following perallylation, but was negligibly alkylated following perbenzylation; 4) when unprotected, the reactive indole nitrogen of tryptophan was alkylated; 5) the use of 2-bromo-Cbz protection for tyrosine resulted in formation 30 of the methyl and allyl ether analogs and any O-benzyl products formed using benzyl bromide in the alkylation

were cleaved during the hydrogen fluoride treatment; and
6) when tyrosine hydroxyl was t-Bu protected, the side
chain was unmodified. Although not studied in detail,
glutamic acid t-Bu ester led to multiple products
5 following repeated alkylations. Other amino acid
derivatives having side chains with potentially reactive
functionalities, including serine, threonine,
hydroxyproline (all protected as their t-Bu ether),
methionine (sulfoxide), and tryptophan (Boc), did not
10 undergo any modification during the alkylation step.
Repetitive alkylations of trityl-protected N-terminal
glycine and β -alanine led to side products containing
additional alkyl groups as detected by MALDI-MS.

The combinatorial library of compounds of
15 Formula I (wherein B=0) has an OOX format, where O
represents a defined position and X represents a mixture
position. Forty-six different amino acids (cysteine and
histidine were excluded since analogs containing these
amino acids were found to have significant side reactions
20 and/or incomplete reaction during the alkylation
procedure) were incorporated into the first X position
(R₃), and 50 different amino acids were incorporated into
the first O position (R₁). The amide alkyl groups in the
second X (R₄) and second O positions (R₆) were: methyl,
25 ethyl, allyl, benzyl or naphthylmethyl. This
combinatorial library consists of 250 mixtures (50 amino
acids \times 5 alkyl groups), each of which is composed of 230
compounds (46 amino acids \times 5 alkyl groups), and was
prepared applying the divide, couple and recombine
30 process, also independently reported as the "mixing and

portioning" and "split synthesis" approaches (Lam, K.S., et al., Nature, 354:82 (1991); Furka, A., et al., Int. J. Pept. Protein Res., 37:487 (1991)). The stepwise synthesis was carried out on the solid phase by

5 alternating amino acid attachment and alkylation of the previously formed amide bond as outlined in Figure 1. Standard Fmoc chemistry for the incorporation of amino acids was used with MBHA resin as the solid support.

Alkylation of the amide bond between the C-terminal amino

10 acid and the MBHA linker was found to significantly decrease the stability of the amide-resin linkage to acidolytic conditions. (Kornreich, W., et al., Int. J. Pept. Protein Res., 25:414 (1985)). The amino groups were protected with the bulky trityl group to avoid

15 modification of the N-terminal amine during the manipulation of the amide groups of the resin-bound compounds. The five alkyl halides [methyl iodide, ethyl iodide, allyl bromide, benzyl bromide, and 2-(bromomethyl)naphthalene] were reacted with the

20 previously formed amide anions using repeated treatments of the alkylation method described above. Replicates of control resins Trt-Leu-MBHA and Trt-Trp-MBHA were added during each of the five separate alkylation treatments on the solid phase resins. The R₈ residues were introduced

25 at the same time, enabling the completeness of each of these reactions to be determined. A second amino acid derivative was then coupled to these control resins following removal of the trityl group with 2% trifluoroacetic acid in dichloromethane. This resulted

30 in the generation of individual compounds having the formulas H₂N-Phe-Leu-NHR and H₂N-Ala-Trp(R)-NHR (R =

methyl, ethyl, allyl, benzyl, or naphthylmethyl). No starting material was detected by RP-HPLC for the crude compounds following three treatments with methyl iodide and ethyl iodide. Allylation, benzylation and 5 naphthylmethylation required six repetitions of the alkylation procedure, with generally less than 10% starting material remaining (as determined by RP-HPLC). In case of the Ala-Trp controls up to 40% of monoalkylated material was seen (using RP-HPLC). The 230 10 resins containing the first two library positions were then combined, thoroughly mixed and divided into 250 equal portions (50×5 library resin packets). Following trityl removal, the second group of protected amino acids was added (cysteine and histidine included), the Fmoc 15 group was removed, and the free amino groups were again reacted with trityl chloride. The newly formed amide bond was then alkylated as described above, with the exception that five repetitions of the alkylation procedure were carried out. For this second alkylation 20 step, control resins were prepared having the formula Trt-Phe-Leu-NHMe-MBHA and Trt-Ala-Trp-NHMe-MBHA. These control resins were permethylated at the first amide position to determine the completeness of the second alkylation. Following trityl removal, starting material 25 was not detected by RP-HPLC or MALDI-MS for any of the five crude alkylation control products. The highly acid labile amide linkage between the peptidomimetic and the MBHA resin linker does not permit the acid labile side chain protecting groups to be removed prior to final 30 cleavage from the resin. Thus, the mixtures were cleaved from the resin under standard high hydrogen fluoride

cleavage conditions (Houghten, R.A., et al., Int. J. Pept. Protein Res., 27:673 (1986)) and obtained as lyophilized powders following extraction with 50% aqueous acetonitrile. The yields of some of the crude control 5 compounds were found to be sequence-dependent. During the final acidic Trt removal, compounds having bulky alkyl residues in position R₂ were partially cleaved from the resin. (Gisin, B.F., et al., J. Am. Chem. Soc., 94:3102 (1972)).

10 Compounds of the formula H₂N-Phe-N(R)-Leu-NHMe (R = methyl, ethyl, allyl, benzyl or naphthylmethyl) were individually synthesized using the described method to provide material as analytical controls. Following purification by preparative RP-HPLC, the identity of each 15 compound was confirmed by RP-HPLC, MALDI-MS, HR-MS, microanalysis, and NMR.

The nonsupport-bound library mixtures were screened in solution in radio-receptor, antimicrobial and enzyme inhibition assays. Deconvolution of the highly 20 active mixtures was carried out by both iterative and positional scanning methods. The iterative method is set forth in Dooley, et al., Science, 266:2019-2022 (1994) and the positional scanning method is set forth in U.S. Patent Application Serial No. 07/943,709, herein 25 incorporated by reference.

The immediately preceding description sets forth in general the reaction techniques utilized in synthesizing the selectively N-alkylated compounds of

Formula I. These techniques can be utilize in one of two strategic approaches for finding the most active compound; the iterative approach or the positional scanning approach. The iterative approach is well-known and is set forth in general in Houghten et al., Nature, 354, 84-86 (1991); and Dooley et al., Science, 266, 2019-2022 (1994); herein incorporated by reference. In the iterative approach, for example, sublibraries of a molecule having six variable groups are made wherein the first two variables are defined. (With reference to Figure, an example for this discussion would have B is 1, R₁₀ is absent, R₁ and R₂ are each a hydrogen atom, with R₃ through R₆ as the six variable groups.) Each of the compounds with the two defined variable groups is reacted with all of the other possibilites at the other four variable groups. These sub-libraries are each tested to define the identity of the third varible in the sub-library having the highest activity in the screen of choice is determined. A new sub-library with the first three variable poisitions defined is reacted again with all the other possibilities at the remaining three undefined variable positions. As before, the identity of the fourth variable position in the sub-library having the highest activity is determined, and a new set of sub-libraries, with four defined variable regions, is synthesized. This process is repeated for all six variables, yielding the compound with each variable contributing to the highest desired activity in the screening pocess. Promising compounds from this process can then be synthesized on larger scale in traditional

single-compound synthetic methods for further biological investigation.

The positional-scanning approach has been

- 5 described for various organic libraries and for various peptide libraries (see, for example, R. Houghten et al. PCT/US91/08694, S.P.A. Fodor and L. Stryer and U.S. Patent Application No. 07/876,792, herein incorporated by reference). However, the positional-scanning approach
10 has never been applied to the selectively N-alkylated compounds of the instant Formula I. In the positional scanning approach sublibraries are made defining only one variable with each set of sublibraries- and all possible sublibraries with each single variable defined (and all
15 other possibilities at all of the other variable positions) is made and tested. From the instant description one skilled in the art could synthesize libraries wherein 2 fixed positions are defined at a time. From the testing of each single-variable defined
20 library, the optimum substituent at that position is determined, pointing to the optimum or at least a series of compounds having a maximum of the desired biological activity. As this approach is applied to the selectively N-alkylated compounds of Formula I, sublibraries where
25 each possibility of any one R group (e.g. R₈) is defined and all other possibilities of the remaining R groups are synthesized and screened for the desired activity. Thus, the number of sublibraries for compounds with a single position defined will be the number of different
30 substituents desired at that position, and the number of all the compounds in each sublibrary will be the product

of the number of substituents at each of the other variables. Thus, the instant invention is directed to screening sublibraries of the selectively N-alkylated compounds of Formula I wherein each sublibrary has an R 5 group defined, and all other R groups are synthesized with the desired substituents, and defining each single variable in a similar grouping of sublibraries and screening for biological activity, until all such variable positions have been defined and screened for the 10 desired activity. One skilled in the art would realize that this approach could also be applied in the situation wherein each sublibrary has two R groups defined, using a modification of the above techniques.

15 The reduction of the interior amide of the compounds of Formula I is another means for the chemical transformation of such compounds which adds stability and can enhance activity. A number of reagents are available and well known for the reduction of amides to 20 amines such as those disclosed in Wann et al., JOC, 46:257 (1981) and Raucher et al., Tett.Let., 21:14061 (1980), both of which are incorporated herein by reference. Diborane has the advantage that trimethylborate, the only by-product in the reaction 25 workup, is volatile and is therefore readily removed by evaporation in solution phase reduction. The use of excess diborane in refluxing tetrahydrofuran permits simple aliphatic and aromatic amides to be rapidly, and often quantitative be reduced into their corresponding 30 amines.

A newly synthesized compound can be purified using a method such as reverse phase high performance liquid chromatography (RP-HPLC) or other methods of separation based on the size or charge of the compound.

- 5 Furthermore, the purified compound can be characterized using these and other well known methods such as amino acid analysis and mass spectrometry.

After manufacture, the compounds can be assayed for receptor binding activity using the radioreceptor 10 assay (Examples III and IV) or other assays outlined below, including the glycosidase assay (Example V).

Because some of the compounds of the present invention bind to the μ receptor, they can be used in in vitro assays to study the opiate receptor subtypes. For 15 example, in a sample receptor of unknown type or origin, the compounds, after being labeled with a detectable marker such as a radioisotope, can be contacted with the receptor sample under conditions which specifically favor binding to a particular receptor subtype. Unbound 20 receptor and compound can be removed, for example, by washing with a saline solution, and bound receptor can then be detected using methods well known to those skilled in the art. Therefore, the compounds of the present invention are useful in vitro for the diagnosis 25 of relevant opioid receptor subtypes, and in particular the μ type, in brain and other tissue samples.

In addition to their utility in in vitro screening methods, the compounds are also useful in vivo.

For example, certain of the instant compounds can be used in vivo diagnostically to localize opioid receptor subtypes. The compounds are also useful as drugs to treat pathologies associated with other compounds which 5 interact with the opioid receptor system. It can be envisioned that these compounds can be used for therapeutic purposes to block the peripheral effects of a centrally acting pain killer. For instance, morphine is a centrally acting pain killer. Morphine, however, has a 10 number of deleterious effects in the periphery which are not required for the desired analgesic effects, such as constipation and pruritus (itching). While it is known that the many peptides do not readily cross the blood-brain barrier and, therefore, elicit no central effect, 15 the subject peptides can have value in blocking the periphery effects of morphine, such as constipation and pruritus.

This invention provides pharmaceutical compositions comprising the compounds of Formula I in a 20 pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various 25 types of wetting agents.

Suitable pharmaceutical carriers and their formulations are described in Martin, REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed. (Mack Publishing Co., Easton 1975). Such compositions will, in general,

contain an effective amount of the active reagent together with a suitable amount of carrier so as to prepare the proper dosage form for proper administration to the subject.

5 Useful pharmaceutical carriers for the preparation of the pharmaceutical compositions can be solids, liquids or gases. Thus, the compositions can take the form of tablets, pills, capsules, powders, enterically coated or other protected formulations (such
10 as by binding on ion exchange resins or other carriers, or packaging in lipid protein vesicles or adding additional terminal amino acids), sustained release formulations, solutions (e.g. ophthalmic drops), suspensions, elixirs, aerosols, and the like. Water,
15 saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly (when isotonic) for injectable solutions. The carrier can be selected from various oils including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil,
20 soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride,
25 dried skim milk, glycerol, propylene glycol, water, ethanol, and the like.

The compositions may be subjected to conventional pharmaceutical procedures such as sterilization and may contain conventional pharmaceutical

additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, buffers, and the like.

This invention provides methods of effecting
5 treating a mammal comprising the step of administering a therapeutically effective amount of a pharmaceutical composition of this invention to a subject. As used herein, the term "therapeutically effective amount" is that amount necessary to alleviate the condition from
10 which the mammal suffers.

In the practice of the therapeutic methods of the present invention, an effective amount of a pharmaceutical composition of a compound of Formula I is administered via any of the usual and acceptable methods
15 known in the art, either singly or in combination with another compound of the present invention. These compounds or compositions can thus be administered orally, sublingually, topically (e.g., on the skin or in the eyes), parenterally (e.g., intramuscularly,
20 intravenously, subcutaneously or intradermally), or by inhalation, and in the form of either solid, liquid or gaseous dosage including tablets, suspensions, and aerosols, as is discussed in more detail above. The administration can be conducted in single unit dosage
25 form with continuous therapy or in single dose therapy ad libitum.

In one embodiment, the therapeutic methods of the present invention are practiced when the relief of

symptoms is specifically required or perhaps imminently so. In another embodiment, the method is effectively practiced as continuous or prophylactic treatment.

In the practice of the therapeutic methods of
5 the invention, the particular dosage of pharmaceutical
composition to be administered to the subject will depend
on a variety of considerations including the nature of
the disease, the severity thereof, the schedule of
administration, the age and physical characteristics of
10 the subject, and so forth. Proper dosages may be
established using clinical approaches familiar to the
medicinal arts. It is presently believed that dosages in
the range 0.1 of 100 mg of a compound of this invention
per kilogram of subject body weight will be useful, and a
15 range of 1 to 100 mg per kg generally preferred where the
administration is by injection or ingestion. Topical
dosages may utilize formulations containing active
peptides and a liquid carrier or excipient, with multiple
daily applications being appropriate.

20 Fluorenylmethoxycarbonyl (Fmoc) amino acid
derivatives were purchased from Calbiochem-Novabiochem
Corp. (San Diego, CA, USA), Bachem Bioscience Inc.
(Philadelphia, PA, USA) and Bachem California (Torrance,
CA, USA). MBHA resin, (1% divinylbenzene, 100 - 200
25 mesh, 0.9 mmol/g substitution), was received from
Peninsula Laboratories, Inc (Belmont, CA, USA). N,N'-
Diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole
(HOBT) were purchased from Chem Impex International (Wood
Dale, IL, USA), trifluoroacetic acid from Halocarbon

(River Edge, NJ, USA) and hydrogen fluoride from Air Products (San Marcos, CA, USA). All other reagents and anhydrous solvents (DMSO, THF) were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). The 5 solvents dichloromethane (DCM), dimethylformamide (DMF), isopropanol (IPA), and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All reagents and solvents were used without further purification. MALDI-MS analyses were carried out on a Kratos Analytical 10 Compact MALDI II (Ramsey, NJ, USA). HR-FAB-MS were recorded at the University of California Riverside Mass Spectrometry Facility, Department of Chemistry (Riverside, CA, USA) on a ZAB mass spectrometer. Analytical RP-HPLC was performed on a Beckman System Gold 15 instrument (Beckman Instruments, Fullerton, CA, USA). Samples were analyzed using Vydac 218TP54 C₁₈ columns (0.46 × 25 cm). Preparative RP-HPLC purification was performed on a Waters Delta Prep 3000 instrument (Millipore, Waters Division, San Francisco, CA, USA). 20 Samples were purified using Waters Delta-Pak C₁₈ columns (2.5 × 10 cm). All gradients reported were linear in eluent A (0.05% TFA aqueous) and eluent B (0.05% TFA in acetonitrile); flow rates were 1 mL/min (analytical) and 20 mL/ min (preparative); the eluent was monitored at 214 25 nm. Routine ¹H NMR and ¹³C NMR spectra were recorded on a Varian Gemini 200 (200 MHz). Microanalyses were performed at Galbraith Laboratories, Inc. (Knoxville, TN, USA).

Library synthesis**Amino acid derivatives**

The following amino acid derivatives were used in synthesizing a combinatorial library according to

- 5 Formula I above: Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Gly-OH,
Fmoc-Ile-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Met(O)-OH,
Fmoc-Asn-OH, Fmoc-Pro-OH, Fmoc-Gln-OH, Fmoc-Arg(Pmc)-OH,
Fmoc-Ser(t-Bu)-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Val-OH,
10 Fmoc-Trp(Boc)-OH, Fmoc-Trp-OH, Fmoc-Tyr(2BrCbz)-OH,
Fmoc-Tyr(t-Bu)-OH, Fmoc-D-Ala-OH, Fmoc-D-Phe-OH,
Fmoc-D-Ile-OH, Fmoc-D-Lys(Boc)-OH, Fmoc-D-Leu-OH, Fmoc-D-Asn-OH,
Fmoc-D-Pro-OH, Fmoc-D-Gln-OH, Fmoc-D-Ser(t-Bu)-OH,
Fmoc-D-Thr(t-Bu)-OH, Fmoc-D-Val-OH, Fmoc-D-Trp(Boc)-OH,
15 Fmoc-D-Trp-OH, Fmoc-D-Tyr(t-Bu)-OH, Fmoc-D-Arg(Pmc)-OH,
Fmoc-L-Nle-OH, Fmoc-D-Nle-OH, Fmoc-L-Nve-OH, Fmoc-D-Nve-OH,
Fmoc-L-Nal-OH, Fmoc-D-Nal-OH, Fmoc-L-Phg-OH,
Fmoc-L-Glu(t-Bu)-OH, Fmoc-D-Glu(t-Bu)-OH, Fmoc- β -Ala-OH,
Fmoc-L-Cha-OH, Fmoc-D-Cha-OH, and Fmoc-Hyp(t-Bu)-OH.

Fmoc-Cys(MeOBn)-OH (MeOBn = 4-methoxybenzyl),

- 20 Fmoc-Cys(MeBn)-OH (MeBn = 4-methylbenzyl), Fmoc-His(Trt)-OH and Fmoc-D-His(Trt)-OH were also used in the N-terminal position of the library.

EXAMPLE I**A. Synthesis of the combinatorial library****1. Coupling of the first amino acid derivative**

The library described below was synthesized
5 using simultaneous multiple peptide synthesis (Houghten,
R.A., Proc. Natl. Acad. Sci. USA, 82:5131 (1985). The
solid support (MBHA resin) was contained in 230
polypropylene mesh packets (250 mg resin per packet;
packet size 3 cm x 3 cm). For use in the synthesis of
10 control compounds, 40 additional polypropylene mesh
packets were prepared containing MBHA resin (100 mg).

After the common wash and neutralization steps
were carried out (1 x DCM, 2 x 5% DIEA, 2 x DCM, 2 x DMF;
approximately 8 mL per packet; all resin packets were
15 completely covered with solvent) on all of the resin
packets, the individual resin packets were separated into
46 groups, each containing five packets for the addition
of the 46 amino acid derivatives used in the first
coupling step. Fmoc-Leu-OH and Fmoc-Trp-OH were added to
20 two groups of 20 control resin packets. Amino acid
couplings were carried out on each of the 46 groups of
five library resin packets by vigorously shaking 44
groups in a solution (67.5 mL) of 0.1 M Fmoc amino acid
derivative (6.75 mmol)/DIC/HOBt in DMF overnight (Fmoc-
25 Gly coupling required only 75 min); for the other two
groups, library resin packets and control packets were
vigorously shaken in a solution (175.5 mL) of 0.1 M Fmoc-

L-Leu (17.55 mmol)/DIC/HOBt and 0.1 M of Fmoc-L-Trp (17.55 mmol)/DIC/HOBt in DMF overnight. The resin packets were washed (2 x DMF, 1 x DCM, 1 x MeOH; approximately 8 mL per packet) and the completeness of 5 amino acid coupling was verified using the ninhydrin test (Kaiser, E.T., et al., Anal. Biochem., 34:595 (1970)). The only amino acids which required repetitive couplings were Fmoc-L-Gln-OH, Fmoc-D-Gln-OH, Fmoc-L-Arg(Pmc)-OH and Fmoc-D-Lys(Boc)-OH. Removal of the Fmoc protecting group 10 was accomplished by shaking the resin packets in 20% piperidine/DMF (1 x 3 min, 1 x 10 min; 2 L) followed by a wash cycle (5 x DMF, 2 x IPA, 3 x DCM; approximately 8 mL per packet).

2. Tritylation of the N-terminal amino group

15 Following removal of the Fmoc group, the 270 resin packets (a total of 55 mmol of free N- α -amino groups) were shaken for 3 h in a 0.077 M solution of trityl chloride (276.75 mmol) in DCM/DMF (9:1, 3.6 L) containing diisopropylethylamine (DIEA, 1.6 mol, 280 mL). 20 After a short wash procedure (1 x DMF, 1 x 5% DIEA, 1 x DCM; approximately 8 mL per packet), the tritylation procedure was repeated twice more by shaking overnight in a 0.05 M solution of trityl chloride in DCM (5.5 L) containing the same amount of base and washed (2 x DMF, 1 x 5% DIEA, 3 x DCM, 1 x MeOH; approximately 8 mL per 25 packet). The completeness of the trityl coupling was verified for each of the 46 different amino acid resins using the bromophenol blue color test (Krchňák, V., et al., Coll. Czech. Chem. Comm., 53:2542 (1988)).

3. Alkylation of the first amide position

All manipulations were performed under a nitrogen atmosphere and strictly anhydrous conditions.

The 270 resin packets were dried overnight at 50 mTorr.

- 5 Each of five groups, containing 46 amino acid resin packets plus control resin packets (including four Trt-Leu-MBHA packets and four Trt-Trp-MBHA packets), were placed in one of five separate round-bottom flasks - one for each of the five alkylation reactions. Each flask
- 10 contained the same amount of available amide groups (11.07 mmol each). 1 M lithium t-butoxide in THF (220 mmol, 220 mL) and THF (220 mL) were added to each of the five reaction vessels and shaken at room temperature for 15 min. Excess base solution was removed by cannulation.
- 15 Following addition of DMSO (440 mL), the individual alkylating agent was added (665 mmol i.e., 41.4 mL methyl iodide; 53.1 mL ethyl iodide; 57.5 mL allyl bromide; 79.0 mL benzyl bromide). 2-(Bromomethyl)naphthalene (665 mmol, 147 g) was dissolved in DMSO (440 mL) and
- 20 transferred as a solution to the reaction vessel. The reaction mixture was vigorously shaken for 2 h at room temperature. The alkylation solution was removed by cannulation and the entire procedure repeated twice more. The resulting resin packets were washed (3 x DMF, 2 x
- 25 IPA, 3 x DCM, 1 x MeOH; approximately 8 mL per packet) and dried. Following complete drying of the resin packets overnight at 50 mTorr, the process described above was repeated three times for allylation, benzylation and naphthylmethylation (each alkylation, 2 x
- 30 2 h and 1 x 5 h).

4. Recombine, mix and divide the resin

The resin of the 230 library packets was combined, mixed in DCM (2 L; 15 h shaking), and dried. The resin was divided into 250 polypropylene mesh packets 5 (packet size 3 cm x 3cm; each containing 310 mg resin).

5. Removal of the trityl protecting group

The resin packets, prepared as described above, were washed (1 x DCM; approximately 8 mL per packet), treated twice with 2% TFA in DCM (1 x 2 min, 1 x 30 min; 10 2 L), and washed (1 x DCM, 2 x IPA, 2 x DCM, 1 x MeOH; approximately 8 mL per packet).

6. Coupling of the second amino acid derivative and second alkylation

The amino acid coupling (using the 50 different 15 amino acid derivatives), Fmoc removal, tritylation of the free amino groups, alkylation of the previously formed amide bond and trityl removal were performed as described above. Trt-Phe-Leu-NMe-MBHA resin packets and Trt-Ala-Trp(Me)-NMe-MBHA resin packets were added as control 20 resins during alkylation. The second amide position was treated five times for alkylation (methylation and ethylation, each 5 x 2 h; allylation, benzylation and naphthylmethylation, each 3 x 2 h and 2 x 3 h).

7. HF cleavage

The 250 mixture resin packets were cleaved 24 at a time with hydrogen fluoride (5 mL per resin packet with 0.35 mL anisole added as scavenger) using a multiple 5 vessel cleavage apparatus (Kornreich, W., et al., Int. J. Pept. Protein Res., 25:414 (1985)). The resulting mixtures were extracted by sonicating with 50% aqueous acetonitrile (3 x 5 ml). The resulting solutions were lyophilized and relyophilized twice more from 50% aqueous 10 acetonitrile.

8. Individual compounds

Individual compounds were prepared in the same manner as described for the library synthesis. The alkylations were generally performed with repetitions. 15 Following HF cleavage, the crude individual compounds were purified by preparative RP-HPLC. Condition for preparative HPLC: vydac C18; linear gradient 25-55% B, in 30 min; eluent A: 0.05% TFA aqueous; eluent B: 0.05% TFA in acetonitrile; flow rate: 20 mL/min; the eluent was 20 monitored at 214 nm.

Phenylalanyl-N-methyl-leucinemethylamide. Yield after preparative HPLC (TFA salt): 59.8%. ¹H NMR (200 MHz, CDCl₃; mixture of conformers; selected data for the major conformer; ratio 78 : 22): δ = 0.85 (m; 6H), 1.23 - 1.57 25 (m; 2H), 1.71 - 1.85 (m; 1H), 2.65 (s; 3H), 2.7 (d; 3H), 3.01 - 3.28 (m; 2H), 4.51 - 4.58 (m; 2H), 6.85 (m; 1H), 7.15 - 7.28 (m; 5H), 8.5 (br; 2H).

¹³C NMR (200 MHz, CDCl₃; selected data for major conformer): δ = 22.0, 22.7, 24.7, 26.1, 32.6, 36.8, 37.4, 51.7, 57.8, 128.1, 129.1, 129.4, 133.4, 168.9, 170.1. MALDI-MS: 307 (M+2), 329 (M+Na). Anal. calcd. 5 for C₁₉H₂₈F₃N₃O₄ (TFA salt): C, 54.39; H, 6.73; N, 10.02. Found: C, 54.19; H, 7.03; N, 9.99. HR-FAB-MS calcd. for C₁₇H₂₈N₃O₂ (MH⁺) 306.2175, found 306.2165.

Phenylalanyl-N-ethyl-leucinemethylamide. Yield after preparative HPLC (TFA salt): 18.8%. ¹H NMR (200 MHz, 10 CDCl₃; selected data for major conformer; ratio 72 : 28): δ = 0.75 - 1.15 (m; 9H), 1.35 - 1.60 (m; 2H), 1.95 - 2.20 (m; 1H), 2.66 (d; 3H), 2.85 - 3.45 (m; 4H), 3.97 (m; 1H), 4.41 (m; 1H), 6.99 (m; 1H), 7.18 - 7.40 (m; 5H), 8.2 - 9.2 (br; 2H). ¹³C NMR (200 MHz, CDCl₃; selected data for 15 major conformer): δ = 14.3, 22.9, 23.1, 25.7, .26.6, 38.3, 43.5, 52.2, 58.3, 128.6, 129.6, 130.1, 134.1, 169.3, 171.2. MALDI-MS: 321 (M+1), 343 (M+Na). Anal. calcd. for C₂₀H₃₀F₃N₃O₄ (TFA salt): C, 55.399; H, 6.9789; N, 9.697. Found: C, 54.39; H, 6.97; N, 9.47. HR-FAB-MS 20 calcd. for C₁₈H₃₀N₃O₂ (MH⁺) m/z = 320.2331, found m/z = 320.2335.

Phenylalanyl-N-allyl-leucinemethylamide. Yield after preparative HPLC (TFA salt): 20.05%. ¹H NMR (200 MHz, CDCl₃; selected data for major conformer; ratio 71 : 29): 25 δ = 0.76 - 0.90 (m; 6H), 1.27 - 1.50 (m; 2H), 1.98 - 2.08 (m; 1H), 2.68 (d; 3H), 3.02 - 3.60 (m; 4H), 4.18 (m; 1H), 4.41 - 4.49 (m; 1H), 5.12 - 5.24 (m; 2H), 5.56 - 5.78 (m; 1H), 6.99 (m; 1H), 7.21 - 7.36 (m; aromatic protons), 8.35 - 9.35 (br; 2H). ¹³C NMR (200 MHz, CDCl₃; selected

data for major conformer): δ = 22.3, 25.0, 26.0, 37.8, 49.9, 51.8, 58.0, 119.9, 128.1, 129.1, 129.5, 132.1, 133.5, 169.1, 170.4. MALDI-MS: 333 (M+1), 355 (M+Na). Anal. calcd. for $C_{21}H_{30}F_3N_3O_4$ (TFA salt): C, 56.60; H, 6.79; N, 9.44. Found: C, 56.00; H, 6.83; N, 9.23. HR-FAB-MS calcd. for $C_{19}H_{30}N_3O_2$ (MH^+) m/z = 332.2331, found m/z = 332.2335.

Phenylalanyl-N-benzyl-leucinemethylamide. Yield after preparative HPLC (TFA salt): 20.34%. 1H NMR (200 MHz, $CDCl_3$; selected data for major conformer; ratio 65 : 35): δ = 0.72 - 0.89 (m; 6H), 1.02 - 1.53 (m; 2H), 1.87 - 2.11 (m; 1H), 2.52 (d; 3H), 2.98 - 3.45 (m; 2H), 4.05 - 4.79 (m; 4H), 6.92 (m; 1H), 7.08 - 7.38 (m; 10H), 8.20 - 9.20 (br; 2H). ^{13}C NMR (200 MHz, $CDCl_3$; selected data for major conformer): δ = 22.0, 22.9, 25.5, 26.0, 37.7, 38.1, 47.3, 52.5, 57.9, 127.2, 127.7, 128.2, 129.3, 129.8, 133.5, 134.9, 169.7, 170.6. MALDI-MS: 383 (M+1), 405 (M+Na). Anal. calcd. for $C_{25}H_{32}F_3N_3O_4$ (TFA salt): C, 60.58; H, 6.512; N, 8.48. Found: C, 60.33; H, 6.41; N, 8.43. HR-FAB-MS calcd. for $C_{23}H_{32}N_3O_2$ (MH^+) m/z = 382.2487, found m/z = 382.2511.

Phenylalanyl-N-naphthylmethyl-leucinemethylamide. Yield after preparative HPLC (TFA salt): 18.54%. 1H NMR (200 MHz, $CDCl_3$, selected data for major conformer; ratio 67 : 25 : 33): δ = 0.70 - 0.90 (m; 6H), 1.15 - 1.58 (m; 2H), 1.94 - 2.13 (m; 1H), 2.44 (d; 3H), 3.04 - 3.49 (m; 2H), 4.20 - 4.90 (m; 4H), 6.90 - 6.99 (m; 1H), 7.08 - 7.89 (m; 12H), 8.1 - 9.5 (br; 2H). ^{13}C NMR (200 MHz, $CDCl_3$; selected

data for major conformer): δ = 22.0, 23.0, 25.5, 25.7, 37.7, 38.1, 52.6, 57.7, 124.6 - 134.2 (aromatic carbons), 169.8, 170.5. MALDI-MS: 433 (M+1), 455 (M+Na). Anal. calcd. for $C_{29}H_{34}F_3N_3O_4$ (TFA salt): C, 63.82; H, 6.28; N, 5 7.70. Found: C, 63.96; H, 6.27; N, 7.76. HR-FAB-MS calcd. for $C_{27}H_{34}N_3O_2$ (MH^+) m/z = 432.2643, found m/z = 432.2663.

EXAMPLE II

A. Synthesis of the resin bound peptidomimetic compound

10 $H_2N\text{-Tyr}(tBu)\text{-N(Me)\text{-Tyr}(tBu)\text{-N(Bzl)\text{-resin}}$

1. Coupling of the first amino acid derivative

The peptidomimetic compound was synthesized using simultaneous multiple peptide synthesis (Merrifield, R. B. J. Am. Chem. Soc. 1963, 85, 2149; Houghten, R. A. 15 Proc. Natl. Acad. Sci. USA 1985, 82, 5131) and Fmoc strategy. The solid support (MBHA resin) was contained in a polypropylene mesh packet (100 mg resin per packet; packet size 3 cm x 3 cm).

After the neutralization and wash steps were 20 carried out [1 x DCM, 2 x 5% N,N-diisopropylethylamine (DIEA), 2 x DCM, 2 x DMF; approximately 5mL for each washing step] the resin packet was vigorously shaken in a solution (5.4 mL) of 0.1 M Fmoc-L-Tyr(tBu)-OH (0.54 mmol)/DIC/HOBt in DMF overnight. The resin packet was 25 washed (2 x DMF, 1 x DCM, 1 x MeOH) and the completeness of amino acid coupling was verified using the ninhydrin

test (Kaiser, E. T.; Colescott, R. L.; Blossinger, C. D.; Cook, P. I. *Anal. Biochem.* 1970, 34, 595). One repetitive coupling was required. Removal of the Fmoc protecting group was accomplished by shaking the resin 5 packet in 20% piperidine/DMF (1 x 3 min, 1 x 10 min; 2 L) followed by a wash cycle (5 x DMF, 2 x IPA, 3 x DCM).

2. Tritylation of the N-terminal amino group

The resin packet (0.09 mmol of free N- α -amino groups) was shaken for 2 h in a 0.077 M solution of 10 trityl chloride (0.45 mmol) in DCM/DMF (9:1, 5.84 mL) containing DIEA (2.61 mmol, 0.45 mL). After a short wash procedure (1 x DMF, 1 x 5% DIEA, 1 x DCM), the tritylation procedure was repeated three more times by shaking overnight in a 0.077 M solution of trityl 15 chloride in DCM (5.84 mL), for 3 h in a 0.077 M solution of trityl chloride in DCM/DMF (9:1, 5.84 mL) and again overnight in a 0.05 M solution of trityl chloride in DCM (9 mL), containing the same amount of base. The resin packet was washed (2 x DMF, 1 x 5% DIEA, 3 x DCM, 1 x 20 MeOH) and a small resin sample was tested for the completeness of the trityl coupling using the bromophenol blue color test (Krčňák, V.; Vágner, J.; Šafář, P.; Lebl, M. *Coll. Czech. Chem. Comm.* 1988, 53, 2542).

25 3. Alkylation of the first amide position

All manipulations were performed under a nitrogen atmosphere and strictly anhydrous conditions. The resin packet was dried overnight at 50 mTorr. 1 M

lithium t-butoxide in THF (1.8 mmol, 1.8 mL) and THF (1.8 mL) were added to the reaction vessel containing the resin packet (0.09 mmol amide groups) and it was shaken at room temperature for 15 min. Excess base solution was 5 removed by cannulation. Following addition of DMSO (3.6 mL), benzyl bromide (5.4 mmol, 0.64 mL) was added. The reaction mixture was vigorously shaken for 2 h at room temperature. The alkylation solution was removed by cannulation and the entire procedure repeated twice more.

10 The resulting resin packet was washed (3 × DMF, 2 × IPA, 3 × DCM, 1 × MeOH; approximately 5 mL) and dried. Following complete drying of the resin packet overnight at 50 mTorr, the process described above was repeated again two times.

15 4. Removal of the trityl protecting group

The resin packet was washed (1 × DCM; approximately 5 mL), treated twice with 2% TFA in DCM (1 × 3 min, 1 × 30 min), and washed (1 × DCM, 2 × IPA, 2 × DCM, 1 × MeOH; approximately 5 mL).

20 5. Coupling of the second amino acid derivative and second alkylation

The coupling of Fmoc-L-Tyr(tBu)-OH to the resin bound compound, the Fmoc removal, and the tritylation (only three treatments were required) of the free amino 25 groups were performed as described above.

6. Alkylation of the second amide position

All manipulations and the base treatment were performed as described above. For the alkylation DMSO (3.6 mL) and methyl iodide (5.4 mmol, 0.34 mL) were 5 added. The reaction mixture was vigorously shaken for 2 h at room temperature. The alkylation solution was removed by cannulation and the entire procedure repeated twice more. The resulting resin packet was washed (3 × DMF, 2 × IPA, 3 × DCM, 1 × MeOH; approximately 5 mL) and 10 the trityl protecting group removed as described above.

B. Synthesis of $(\text{CH}_3)_2\text{CH}-\text{NH}-\text{Tyr}(\text{tBu})-\text{N}(\text{Me})-\text{Tyr}(\text{tBu})-\text{N}(\text{Bzl})$ -resin

1. Reductive alkylation

This procedure was adapted from those known in 15 the art: Borch, R.F., et al., J. Am. Chem. Soc., 93:2897 (1971); Coy, D.H., et al., Tetrahedron, 44:835 (1988); Staňková, M., et al., Drug Development Research, 33:146 (1994) (herein incorporated by reference).

Resin packets containing resin bound compounds 20 with free N-terminal amino groups were shaken in a solution of methanol (MeOH) 20%/dichloromethane (DCM) 79%/acetic acid 1% (for one resin packet containing 0.05 mmol amine 4 ml of solvent were used - enough to cover the resin packet) and 2 - 10 equivalents of the aldehyde or 25 ketone (depending on their reactivity). After 20 minutes, 2-10 equivalents of a 1 M solution of sodium

cyanoborohydride in N,N-dimethylformamide (DMF) were added and the reaction mixture shaken for 60 min. The resin packets were washed using the following washing sequence: 5 x DMF, 1 x DCM, 1 x MeOH (for one resin 5 packet of the size mentioned above approximately 5 ml of solvent for each step). The completeness of the formation of secondary amines can be tested using hte Kaiser test. If necessary the reaction can be repeated, also by using a different solvent system like DMF 10 containing 1% acetic acid.

The applicability of this reaction to all amino acid derivatives used in the peptidomimetic library was applied to sets of 50 model dipeptide resins (OL-resins). Following HF cleavage, the model compounds were analyzed 15 by HPLC and MALDI-MS. This procedure was carried out as described in R.F. Borch, *et al.*, *J. Am. Chem. Soc.*, 93:2897-2904 (1971); D.H. Coy, *et al.*, *Tetrahedron*, 44:835-841 (1988); and M. Stankova, *et al.*, *Drug Development Research*, 33:146-156 (1994) (all of which are 20 herein incorporated by reference).

Following neutralization (3 x 5% DIEA, 2 x DCM) and a washing step (1 x DMF/ 2 % acetic acid), the resin packet was shaken in a solution of MeOH 20%/DCM 79%/ acetic acid 1% (4 mL) and acetone (0.9 mmol; 66.6 μ L). 25 After 20 min 0.9 mL (0.9 mmol) of a 1 M solution of sodium cyanoborohydride in DMF were added and the reaction mixture was shaken for 60 min. The resin packet was washed using the following washing sequence: 5 x DMF, 1 x DCM, 1 x MeOH, approximately 5 ml of solvent

for each step. The completeness of the formation of the secondary amines was tested using the Kaiser test [if necessary the reaction can be repeated, also by using a different solvent system like DMF containing 1% acetic acid].

C. Synthesis of red[(CH₂)₂CH-NH-Tyr(tBu)-N(Me)-Tyr(tBu)-NH(Bzl)]; (red = reduced)

1. Reduction

Reduction can either be performed on solid support [procedure A] or in solution [procedure B].

Procedure A:

Into a 50 ml glass tube (teflon-lined cap) were added the resin packet and 310 mg boric acid (5.014 mmol). Under nitrogen atmosphere, 0.5 ml trimethylborate (0.0042 mmol) were added, followed by the addition of 15 ml of 1 M borane-tetrahydrofuran complex (15 mmol). Following cessation of hydrogen evolution, the tube was sealed and heated at 65°C for 100 hr. The tubes were then removed, cooled to room temperature and 2ml methanol were added to quench excess reducing agent. The resin packet was washed with THF (1 x 1 min x 10 ml) and MeOH (4 x 1 min x 10 ml). After drying the resin packet, it was covered with 15 ml piperidine and heated at 65°C for 18 hr. The resin packet was washed with DMF (2 x 1 min x 5ml), DCM (2 x 1 min x 5 ml), MeOH (1 x 1

min x 5 ml), DMF (2 x 1 min x 5ml), DCM (2 x 1 min x 5 ml) and MeOH (1 x 1 min x 5 ml).

Procedure B:

The following procedure was adapted from

- 5 Dooley, C.T., et al., Analgesia, INRC Proceedings, 1:400 (1995) (herein incorporated by reference). Into a 50 ml glass tube (teflon-lined cap) were added the compound (0.09 mmol; two backbone carbonyl groups) and 310 mg boric acid (5.014 mmol). Under nitrogen atmosphere, 0.5
10 ml trimethylborate (0.0042 mmol) were added, followed by the addition of 15 ml of 1 M borane-tetrahydrofuran complex (15 mmol). Following cessation of hydrogen evolution, the tube was sealed and heated at 60°C for 90 hr. The tubes were cooled to room temperature and 5ml
15 methanol were added dropwise to remove excess reducing agent. Excess solvent was removed by immersion of the tube in a 55°C water bath under a constant nitrogen flow (10-15 psi). The compound subsequently underwent successive washes and evaporations with methanol (2 x 5
20 ml). After addition of 2 N hydrogen chloride (3 mL; 6 mmol) in water/MeOH (1:3) the glass tube was sealed and heated at 60°C for 18 h to hydrolyze boron-nitrogen complexes. The tube was removed from heat, MeOH (2 ml) was added and the solvent evaporated.

25 C. HF cleavage - Soluble Compounds

The compound was cleaved from the resin with hydrogen fluoride (5 mL per resin packet with 0.35 mL

anisole added as scavenger) using a multiple vessel cleavage apparatus (Houghten, R. A., et al., Int. J. Pept. Protein Res., 27:673 (1986)). If the compound has been reduced on the solid support, the cleavage time was 5 9 h at 0°C; the nonreduced compound was cleaved in 90 min at 0°C. The resulting compound was extracted by sonicating with 50% aqueous acetonitrile (3 × 5 ml). The resulting solution was lyophilized and relyophilized twice more from 50% aqueous acetonitrile.

10

EXAMPLE III**A. Identification Of Mu Selective Opioid Peptides By A Radioreceptor Assay**

This example describes the identification of individual compounds, either contained within a synthetic 15 combinatorial library mixture or prepared separately, as inhibitors of the μ -selective opioid peptide [³H]-[D-Ala², MePhe⁴, Gly-Ol⁵] enkephalin ([³H]-DAMGO). Individual peptides were identified as capable of inhibiting [³H]-DAMGO by a radioreceptor assay.

20

As detailed below, the compound libraries of the instant invention were screened at a single concentration (0.08 mg/ml) in a radioreceptor assay using rat brain homogenates and [³H]-DAMGO as radioligand. IC₅₀ values were determined for mixtures in the library which 25 significantly inhibited the binding of [³H]-DAMGO.

B. Radioreceptor Assays Selective For The Mu Receptor

Rat and guinea pig brains, frozen in liquid nitrogen, were obtained from Harlan Bioproducts for Science (Indianapolis, IN). Frozen brains were thawed,

5 the cerebella removed and the remaining tissue weighed. Each brain was individually homogenized in 40 ml Tris-HCl buffer (50 mM, pH 7.4, 4°C) and centrifuged (39000 x g) (Model J2-HC; Beckman Instruments, Fullerton, CA) for 10 min at 4°C. The pellets were resuspended in fresh Tris-

10 HCl buffer and incubated at 37°C for 40 min. Following incubation, the suspensions were centrifuged as above, the resulting pellets resuspended in 100 volumes of Tris buffer and the suspensions combined. Membrane suspensions were prepared and used in the same day.

15 Protein content of the crude homogenates ranged from 0.15-0.2 mg/ml as determined using the method described by Bradford (Bradford, Anal. Biochem. 72:248-254 (1976), which is incorporated herein by reference).

Binding assays were carried out in

20 polypropylene tubes. Each tube contained 0.5 ml of membrane suspension, 3 nM of the μ -selective opioid peptide [³H]-DAMGO (specific activity 36 Ci/mmol), 0.08 mg/ml compound mixture and Tris-HCl buffer in a total volume of 0.65 ml. Assay tubes were incubated for 60 min

25 at 25°C. The reaction was terminated by filtration through GF-B filters (Wallac, Inc., Gaithersburg, MD). The filters were subsequently washed with 6 ml Tris-HCl buffer at 4°C. Bound radioactivity was counted on a Beta-plate Liquid Scintillation Counter (Life

100

Technologies, Gaithersburg, MD) and expressed in counts per minute (cpm). Inter- and intra-assay variation standard curves were determined by incubation of [³H]-DAMGO in the presence of 0.13-3900 nM of unlabeled DAMGO.

- 5 Competitive inhibition assays were performed as above using serial dilutions of the peptide mixtures. IC₅₀ values were then calculated using the software GRAPHPAD (ISI, San Diego, CA). IC₅₀ values of less than 1000 nM are indicative of highly active opioid compounds which
 10 bind to the μ receptor, with particularly active compounds having IC₅₀ values of 100 nM or less and the most active compounds with values of less than 10 nM.

In the following Table, the only variable occurs at R₇. Thus, all of the following compounds, in
 15 reference to Formula I, have the following structure: X and Y are taken together to form a carbonyl group, R₁ and R₂ are each a hydrogen atom, R₁₀ is absent, B is zero, AA, BB, and CC are zero, R₆ is ethyl, R₈ is napth-2-ylmethyl, and R₉ is a hydrogen atom.

20

TABLE 1 Mu Receptor Assay		
	R ₇	IC ₅₀ (nM)
	S-methyl	2
	S-(2-(methylsulfinyl)ethyl)	7
25	hydrogen atom	13
	S-(4-hydroxy)benzyl (2-BrZ) ¹	31
	S-(Hydroxy)methyl	40
	S-(4-hydroxybenzyl) (t-butyl) ¹	74

20

TABLE 1
Mu Receptor Assay

	R ₁	IC ₅₀ (nM)
	S-(indol-3-yl)methyl (Boc) ¹	92
	S-(1-methyl)prop-1-yl	129
	S-(3-(N,N,N-triethyl)guanidino)-N-propyl	138
	S-(4-(N-(naphth-2-ylmethyl)amino)-n-butyl	237
5	R-methyl	246
	S-Cyclohexylmethyl	265
	S-Phenyl	384
	S-Benzyl	471
	R-(4-(N-(naphth-2-ylmethyl)amino)-n-butyl)	476
10	S-(2-carboxy)eth-1-yl	476
	S-(N-(naphth-2-ylmethyl)indol-3-ylmethyl)	494
	R-4-(hydroxy)benzyl	542
	S-(N,N-di(naphth-2-ylmethyl)amidoethyl	585
	R-(2-methyl)prop-1-yl	666
15	S-Pyrrolidine (taken in conjunction with R ₈)	891
	R-(n-butyl)	1056
	S-((N,N-di(naphth-2-ylmethyl)amidomethyl))	1106
	R-(Hydroxy)methyl	1106
20	R-Pyrrolidine (takin in conjunction with R ₈)	1115
	S-(n-propyl)	1133
	S-(Naphth-2-yl)methyl	1206
	R-(n-propyl)	1343
25	S-(2-methyl)prop-1-yl	1493
	S-(n-butyl)	1560

20

TABLE 1
Mu Receptor Assay

R,	IC_{50} (nM)
R- (indol-3-yl)methyl (Boc) ¹	1593
R- (3- (N,N,N-triethyl)guanidino)-N-propyl)	1630

¹ Protecting group removed before testing

EXAMPLE IV

5 A. Assay for Kappa Opiate Receptor Inhibition

This example demonstrates the specificity of the novel selectively N-alkylated compounds of Formula I for the kappa opiate receptors.

Assays demonstrating selective inhibition of binding to kappa opiate receptors for κ receptors were carried out using [³H]-U69,593 (3 nM, specific activity 62 Ci/mmol) as the radioligand and tissue homogenates prepared from guinea pig brains (cortex and cerebellum) using Tris buffer containing 100 μ M PMSF, 5 mM MgCl₂, and 1 mg/ml BSA, pH 7.4. Sample tubes were incubated for 2.5 hr. Standard curves were prepared using 0.05-6300 nM naloxone.

Tritiated ligands, [³H]-DAMGO, [³H]-DPDPE and [³H]-[D-Ser², Leu⁵, Thr⁶]enkephalin ([³H]-DSLET), Abuse (NIDA) repository, as prepared by Multiple Peptide Systems (San Diego, CA), [³H]-U69,593 from Amersham (Arlington Heights, IL) and [³H]-naltrindole from DuPont

NEN Research Products (Los Angeles, CA). The average standard deviation for IC₅₀ values was ±20%.

In the following Table 2, all compounds have either R₁ or R₂ as a hydrogen atom and the other taken together with R₃ to form a pyrrolidine group. X and Y are taken together to form a carbonyl group, B is zero, AA, BB, and CC are zero except where noted, R₆ is benzyl and R₇ is a hydrogen atom. Thus, only R₅ is varied as the compounds are tested in the assay.

10

TABLE 2
Kappa Receptor Assay

15

15

20

25

R ₅	IC ₅₀ (nM)
S-methyl	1
R-methyl	1
hydrogen atom	1
S-(3-guanidino)-N-propyl	2
S-(4-(N-benzylamino)-n-butyl	4
S-(iso-propyl)	7
S-(2-(methylsulfinyl)ethyl)	11
S-(n-propyl)	14
R-(n-propyl)	15
R-(hydroxy)methyl	16
R-(n-butyl)	21
R-(3-guanidino)-N-propyl	22
R-(Naphth-2-yl)methyl	23
S-(hydroxy)methyl	28
R-(n-butyl)	31
R-(4-(N-benzylamino)-n-butyl	42

		TABLE 2 Kappa Receptor Assay
	R_1	IC_{50} (nM)
10	S-Phenyl	50
	S-Benzyl	53
	S-(4-hydroxybenzyl)	56
	R-(1-methyl)propyl	67
5	R-(4-hydroxybenzyl) (t-butyl) ¹	69
	S-(1-methyl)propyl	74
	S-(4-hydroxybenzyl) (t-butyl) ¹	85
	S-(2-methyl)propyl	99
	S-(Cyclohexylmethyl)	102
10	S-(1-hydroxy)ethyl	108
	R-benzyl	146
	R-(iso-propyl)	203
	S-(Naphth-2-ylmethyl)	222
	R-pyrrolidine (taken in conjunction with R ₂)	247
15	R-Cyclohexylmethyl	284
	R-(2-methyl)propyl	291
	S-(indol-3-yl)methyl (Boc) ¹	306
	S-(N,N-dibenzylamido)ethyl	313
20	hydrogen atom (AA=1)	487
	R-(N' (t-butoxycarbonyl)indol-3-ylmethyl)	496
	R-(propionamide)	642
	1-(hydroxy)ethyl	650
	2-(carboxy)ethyl	898
25	R-(indol-3-ylmethyl)	931
	S-(indol-3-ylmethyl)	1277

10

TABLE 2 Kappa Rec ptor Assay	
	R, IC ₅₀ (nM)
	R- (N,N-dibenzylamido)methyl 2430
	R- (2- (carboxy) ethyl) 4688
	S- (4-hydroxy-pyrrolidine) taken in conjunction with R ₈ 8735
5	S-pyrrolidine (taken in conjunction with R ₈) 13533

¹ Protecting group removed before testing

EXAMPLE V

A. α-Glucosidase Inhibitor

10 α-Glucosidases are not only essential to carbohydrate metabolism, but also vital for the processing of various glycoproteins and glycolipids. Inhibitors of these enzymes, in particular of α-glucosidase, are therefore of high therapeutic potential.

15 α-glucosidase inhibitors are potent oral anti-diabetics (Lebovitz, H.E. Drugs, 44(3):21-28 (1992)), and have been implicated in the blocking of microbial infection (Fischer, P.B., et al. J.Virol 69(9):5791-5797 (1995); Rademacher, T.W., IN: Sandler, M. and Smith, J.H. (Eds.),

20 Enzymes as Drugs Vol.2, Oxford University Press, Oxford, 333-343 (1994)) and tumor growth (Pili, R. et al., Cancer Res., 55:2920-2926 (1995)). Most of the known natural and synthetic α-glucosidase inhibitors are sugar analogs, such as pseudooligosaccharides (Bischoff, H.,

Eur.J.Clin.Investig., 24(3):3-10 (1994)), azasugars (Wong, C.H., et al., J.Org.Chem. 60:1492-1501 (1995)), or indolizidine alkaloids (Elbein, A.D., Ann.Rev.Biochem., 56:497-534 (1987)). Glycosidase inhibitors often inhibit 5 more than one glycolytic enzyme (Kajimoto, T., et al., J.Am.Chem.Soc., 113:6187-6196 (1990)).

The results set forth below in Table 3 are obtained from an α -glucosidase inhibition assay performed in a 96-well format using p-nitrophenyl- α -D-glucopyranoside as chromogenic substrate and α -glucosidase from bakers yeast, essentially as described by Haslvorson and Ellias (Biochem. Biophys. Acta, 30:28-40 (1958)). The IC₅₀ values represent the concentration necessary for 50% enzyme inhibition. The most active 10 inhibitors are compounds of Formula I wherein X and Y are taken together to form a carbonyl group, B is zero, AA, BB, and CC are zero except where noted, R₁ is a hydrogen atom, R₆ is benzyl, R₆ is naphth-2-ylmethyl, R₃ is S-(N-(naphth-2-ylmethyl)indol-3-ylmethyl), R₁ and R₂ are each 15 hydrogen, R₁₀ is absent, and R₇ is as set forth in the following Table 3:

15

20

TABLE 3
 α -Glucosidase Inhibition Assay

	R ₇	IC ₅₀ (μ M)
25	R-(4-(N-benzylamino)-n-butyl)	17
	S-(4-(N-benzylamino)-n-butyl)	19
	S-(3-guanidino)-n-propyl)	38
	R-(3-guanidino)-n-propyl)	38

TABLE 3
 α -Glucosidase Inhibition Assay

	S-pyrrolidine (taken in conjunction with R ₈)	141
	S-methyl	167
	Hydrogen atom	167
5	R-(2-methyl)propyl	170
	S-(1-hydroxymethyl)	176
	S-(phenyl)	184
	S-(4-hydroxybenzyl)	190
	R-methyl	199
10	S-benzyl	328
	S-(2-methyl)propyl	356
	S-(indol-3-ylmethyl)	356
	S-(iso-propyl)	356
	R-(2-methyl)prop-1-yl	398
15	S-4-hydroxypyrrolidine (in conjunction with R ₈)	437
	S-(1-hydroxyethyl)	460
	S-[N',N'-dibenzylamido)ethyl])	529
	R-(4-hydroxybenzyl)	540
20	R-(iso-propyl)	552
	R-(N'-benzyl indol-3-ylmethyl)	552
	S-(2-(methylsulfinyl)ethyl)	564
	S-(1-methyl)prop-1-yl	610
	S-(N'-benzyl indol-3-ylmethyl)	632
25	S-(n-propyl)	632
	R-(indol-3-ylmethyl)	667
	S-(cyclohexylmethyl)	667

TABLE 3
 α -Glucosidas Inhibition Assay

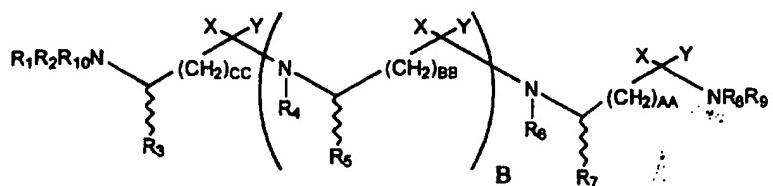
	R' - (1-hydroxyethyl)	678
	R-pyrrolidine (taken in conjunction with R ₈)	702
	S- [N', N'-dibenzylamido)ethyl	713
5	R- (n-butyl)	724
	hydrogen atom, AA=1	770
	R- (n-propyl)	828
	S- (n-butyl)	>1000
	S- (naphth-2-ylmethyl)	>1000
10	R- (naphth-2-ylmethyl)	>1000
	R- (1-hydroxyethyl)	>1000
	S- (2-carboxyethyl)	>1000
	R- (2-carboxyethyl)	>1000
	N', N-dibenzyl R-propionamide	>1000
15	R- (cyclohexylmethyl)	>1000
	R-benzyl	>1000
	S- [(N'N'-dibenzylamido)ethyl	>1000

Numerous modifications and variations are possible in light of the above teachings and, therefore,
20 within the scope of the appended claims, the invention may be practiced otherwise than as particularly described above.

We Claim:

1. A single compound or a library of an approximately equimolar mixture of two or more compounds of the Formula (I):

5



Wherein:

R₁ and R₂ independently are a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₃, R₅, and R₇ are independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, C₁ to C₁₆ substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₄, R₆, and R₈ are independently a C₁ to C₁₈ substituent group; with the proviso that all but one of R₄, R₆ and R₈ can simultaneously be the same group;

R₉ is a hydrogen atom or a solid support;

R_{10} is optionally present as a C₁ to C₁₈ substituent group when R₁ and R₂ are other than a hydrogen atom, an amino protecting group or when both R₁ and R₂ are C₁ to C₁₂ acyl groups;

5 AA, BB, and CC are independently 0 to 5;

B is from 0 to 3;

further wherein the stereochemistry at the carbons bonded to R₃, R₅, and R₇ are independently R or S or a mixture of the two;

10

further wherein when B is 2 or 3; each R₄ and R₅ can be the same or different;

15 with the proviso that either R₁ or R₂ can be taken with R₃; R₄ can be taken with R₅; R₆ can be taken with R₇; respectively and independently, to form a substituted or unsubstituted pyrrolidine ring;

X and Y are either 1) each a hydrogen atom or 2) taken together to represent a carbonyl group;

20 and a pharmaceutically acceptable salt, solvate or hydrate thereof.

2. A single compound of claim 1.

25 3. A single compound of claim 2, wherein X and Y are taken together to form a carbonyl moiety.

4. A single compound of claim 3, wherein B, AA, BB and CC are zero, except that AA can be zero or one when R₁ is a hydrogen atom and that CC can be zero or one when R₃ is a hydrogen atom.

5 5. A single compound of claim 4, wherein R₉ is a hydrogen atom and R₁₀ is absent.

6. A single compound of claim 5 wherein R₃ and R₁ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or 10 R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-(napth-2-ylmethylamino)-n-butyl], S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R- 15 (methylsulfinyl)eth-1-yl, S- or R-acetamido, S- or R-[2-(N,N-dimethyl)acetamido], S- or R-(N, N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or R-(n-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or R-(N-benzyl)acetamido, S- or R-(N,N-di(napth-2- 20 ylmethyl))acetamido, S- or R-(N-(napth-2-ylmethyl))acetamido, S- or R-n-propylamine, S- or R-propionamido, S- or R-(N,N-dimethyl)propionamido, S- or R-(N,N-diethyl)propionamido, S- or R-(N,N-diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, 25 S- or R-(N,N-di(napth-2-ylmethyl)propionamido, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N,N-diallyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-triallyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-trimethyl)-3-guanidino-n-propyl], S- or R-[(N,N,N'-triethyl)-3-

(guanidino)-n-propyl], S- or R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[2-(carboxy)ethyl], S- or R-[iso-propyl], S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(methoxy)benzyl, S- or R-(4-(ethoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-[4-hydroxybenzyl], S- or R-(n-butyl), S- or R-(n-propyl), S- or R-[(naphth-2-yl)methyl], AA is zero or one when R₁ is a hydrogen atom, CC is zero or one when R₂ is a hydrogen atom, S- or R-[cyclohexylmethyl], S- or R-(thiomethyl), or when either R₁ or R₂ are taken together with R₃ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

7. A single compound of claim 6, wherein R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl.

8. A single compound of claim 7, wherein either R₁ or R₂ are each a hydrogen atom, or one of R₁ or R₂ is a hydrogen atom and the other is taken together with R₃ to form an S-pyrrolidine ring.

9. A single compound of claim 8, wherein one of R₁ or R₂ is a hydrogen atom and the other is taken together with R₃ to form an S-pyrrolidine ring.

10. A single compound of claim 9, wherein R₆ is naphth-2-ylmethyl and R₈ is benzyl.

11. A single compound of claim 10, wherein R,
is S- or R-methyl, a hydrogen atom, S- or R-[3-
(guanidino)-n-propyl], S- or R-[4-(N-benzylamino)-n-
5 butyl], S-[iso-propyl], S-[2-(methylsulfinyl)ethyl], S-
or R-(n-propyl), S- or R-(hydroxymethyl), S- or R-[n-
butyl], R-[(naphth-2-yl)methyl], or S-phenyl.

12. A single compound of claim 11, wherein R,
is S- or R-methyl.

10 13. A single compound of claim 8, wherein R₁
and R₂ are each a hydrogen atom and R₃ is an S- benzyl
group.

14. A single compound of claim 13, wherein R₆
is ethyl and R₈ is (naphth-2-yl)methyl.

15 15. A single compound of claim 14, wherein R,
is S-methyl, S-(2-(methylsulfinyl)ethyl), a hydrogen
atom, S-(4-(hydroxy)benzyl) or S-[(hydroxy)methyl].

16. A single compound of claim 15, wherein R,
20 is S-methyl.

17. A single compound of claim 8, wherein R₁
and R₂ are each a hydrogen atom and R₃ is R-[(N-(naphth-2-
ylmethyl)indol-3-yl)methyl].

18. A single compound of claim 17, wherein R₆
25 is naphth-2-ylmethyl and R₈ is benzyl.

19. A single compound of claim 18, wherein R,
is S- or R-[3-(guanidino)-n-propyl] or S- or R-[4-
(benzylamino)-n-butyl].

20. A single compound of claim 7, wherein
5 either R₁ or R₂ is a hydrogen atom or is taken in
conjunction with R₃ to form a pyrrolidine ring, and the
other is C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆
heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C,
to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to
10 C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl
heterocycle.

21. A single compound of claim 2, wherein X
and Y are each a hydrogen atom.

22. A single compound of claim 21, wherein B,
15 AA, BB and CC are zero, except that AA can be zero or one
when R₃ is a hydrogen atom and that CC can be zero or one
when R₃ is a hydrogen atom.

23. A single compound of claim 22, wherein R,
20 is a hydrogen atom and R₁₀ is absent.

24. A single compound of claim 23, wherein R₆
and R₈ are independently methyl, benzyl or 4-
hydroxybenzyl.

25. A single compound of claim 24, wherein R,
25 and R, are independently S-benzyl or S-(4-hydroxybenzyl).

26. A single compound of claim 25, wherein R₈ is benzyl, R₁ is 4-hydroxybenzyl, R₆ is methyl, and R₃ is 4-hydroxybenzyl.

27. A single compound of claim 26, wherein a) R₁ and R₂ are the same and are methyl or a hydrogen atom; b) either R₁ or R₂ is a hydrogen atom and the other is chosen from the group consisting of methyl, iso-propyl, cyclopropylmethyl, 4-hydroxymethyl, N-methylpiperidin-4-yl, and 3-(N,N-dimethylamino)-2-methyl-prop-2-en-1-yl.

10

28. A single compound of claim 25, wherein R₈ is methyl, R₁ is S-benzyl, R₆ is 4-hydroxybenzyl, and R₃ is S-(4-hydroxybenzyl).

29. A single compound of claim 28, wherein R₁ and R₂ are the same and are either a hydrogen atom or methyl, or one of R₁ or R₂ is a hydrogen atom and the other is methyl.

30. A single compound of claim 25, wherein R₈ is methyl, R₁ is S-(4-hydroxymethyl), R₆ is benzyl, and R₃ is S-(4-hydroxybenzyl).

31. A single compound of claim 30, wherein R₁ and R₂ are the same and are either a hydrogen atom or methyl, or one of R₁ or R₂ is a hydrogen atom and the other is methyl.

25 32. An approximately equimolar mixture of two or more compounds of claim 1.

33 An approximately equimolar mixture of two or more compound of claim 32, wherein X and Y are taken together to form a carbonyl group.

5 34. An approximately equimolar mixture of two or more compounds of claim 33, wherein B, AA, BB and CC are zero, except that AA can be zero or one when R, is a hydrogen atom and that CC can be zero or one when R, is a hydrogen atom.

10 35. An approximately equimolar mixture of two or more compounds of claim 34, wherein R, is a solid support and R₁₀ is absent.

15 36. An approximately equimolar mixture of two or more compounds of claim 35, wherein R, and R, are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(t-butoxycarbonylamino)-n-butyl], S- or R-[sec-butyl], S- or R-(methylsulfinyl)eth-1-yl, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N-PMC)-3-(guanidino)-n-propyl], S- or R-(t-butyloxy)methyl, S- or R-[2-(t-butyloxy)ethyl], S-phenyl, S- or R-[3-(t-butoxycarbonyl)-n-propyl], S- or R-[iso-propyl], S- or R-[(N-(t-butoxycarbonyl)indol-3-yl)methyl], S- or R-[4-hydroxybenzyl], S- or R-[(4-(t-butoxy))benzyl], S- or R-(n-propyl), S- or R-(n-butyl), S- or R-[(naphth-2-yl)methyl], S- or R-(3-carboxy-n-propyl), S- or R-(cyclohexylmethyl), S- or R-[(4-methoxybenzylthio)methyl], S-[(4-

methylbenzylthio)methyl], S- or R-thiomethyl, S- or R-[4-(N-methyl-N-(2-butoxycarbonyl)ethyl], S- or R-[4-(N-ethyl-N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[4-(N-allyl-N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[4-(N-benzyl-N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[4-(N-(naphth-2-yl)-N-(t-butoxycarbonyl)amino)-n-butyl], S- or R-[2-(N,N-dimethyl)acetamido], S- or R-(N,N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or R-(n-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or R-(N-benzyl)acetamido, S- or R-(N,N-di(naphth-2-ylmethyl))acetamido, S- or R-(N-(naphth-2-ylmethyl))acetamido, S- or R-(N-propylamine), S- or R-propionamido, S- or R-(N,N-diethyl)propionamido, S- or R-(N,N-diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, S- or R-(N,N-di(naphth-2-ylmethyl))propionamido, S- or R-[(N,N'-diallyl-N-PMC)-3-guanidino-n-propyl], S- or R-[N,N',N'''-triallyl-N-PMC-3-guanidino-n-propyl], S- or R-[(N,N',N'''-trimethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-[(N,N',N'''-triethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(ethoxy)benzyl, S- or R-(4-(methoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-(4-(benzoxy)benzyl, S- or R-(4-Waphth-2-ylmethoxy)benzyl, AA is one or zero when R₁ is a hydrogen atom, CC is one or zero when R₃ is a hydrogen atom, or when either R₁ or R₂ are taken together with R₃, 30 to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

37. An approximately equimolar mixture of two or more compounds of claim 36, wherein R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl.

5 38. An approximately equimolar mixture of two or more compound of claim 37, wherein either R₁ or R₂ is a hydrogen atom or is taken in conjunction with R₃ to form a pyrrolidine ring, and the other is C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ 10 substituted alkyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle.

15 39. An approximately equimolar mixture of two or more compounds of claim 37, wherein either R₁ or R₂ are each a hydrogen atom, or one of R₁ or R₂ is a hydrogen atom and the other is taken together with R₃ to form an S-pyrrolidine ring.

20 40. An approximately equimolar mixture of two or more compounds of claim 34, wherein R₉ is hydrogen and R₁₀ is absent.

41. An approximately equimolar mixture of two or more compounds of claim 40, wherein R₃ and R₄ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-

(naphth-2-ylmethy lamino)-n-butyl], S- or R- [4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(methylsulfinyl)eth-1-yl, S- or R-acetamido, S- or R-[2-(N,N-dimethyl)acetamido], S- or R-(N, N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or R-(n-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or R-(N-benzyl)acetamido, S- or R-(N,N-di(naphth-2-ylmethyl))acetamido, S- or R-(N-(naphth-2-ylmethyl))acetamido, S- or R-propionamido, S- or R-(N,N-dimethyl)propionamido, S- or R-(N,N-diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, S- or R-(N,N-di(naphth-2-ylmethyl))propionamido, S- or R-[3-(guanidino)-n-propyl], S- or R- [(N,N-diallyl)-3-guanidino-n-propyl], S- or R- [(N,N,N'-triallyl)-3-guanidino-n-propyl], S- or R- [(N,N,N'-trimethyl)-3-(guanidino)-n-propyl], S- or R- [(N,N,N'-triethyl)-3-(guanidino)-n-propyl], S- or R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[2-(carboxy)ethyl], S- or R-[iso-propyl], S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(ethoxy)benzyl, 25 S- or R-(4-(methoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-[4-hydroxybenzyl], S- or R-(n-butyl), S- or R-(n-propyl), S- or R-[(naphth-2-yl)methyl], AA is zero or one when R₁ is a hydrogen, Cc is zero or one when R₃ is a hydrogen atom, S- or R-[cyclohexylmethyl], S- or R-[thiomethyl], or when either R₁ or R₂ are taken

together with R₃ to form an S- or R- pyrrolidine or S-[4-(hydroxy)pyrrolidine].

42. An approximately equimolar mixture of two or more compounds of claim 41, wherein R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or napth-2-ylmethyl.

43. An approximately equimolar mixture of two or more compounds of claim 42, wherein R₆ is napth-2-ylmethyl, R₃ is R-[(N- (naphth-2-ylmethyl)indol-3-yl)methyl], and R₁ and R₂ are the same and are each a hydrogen atom.

44. An approximately equimolar mixture of two or more compounds of claim 42, wherein R₆ is ethyl, R₃ is S-benzyl and R₁ and R₂ are the same and are each a hydrogen atom.

45. An approximately equimolar mixture of two or more compounds of claim 42, wherein R₆ is napth-2-ylmethyl, R₃ is S-methyl, R₁ and R₂ are the same and are each a hydrogen atom.

46. An approximately equimolar mixture of two or more compounds of claim 42, wherein either R₁ or R₂ is a hydrogen atom and the other is taken in conjunction with R₃ to form an S-pyrrolidine ring, and R₆ is napth-2-ylmethyl.

47. An approximately equimolar mixture of two or more compound of claim 32, wherein X and Y are the same and are each a hydrogen atom.

48. An approximately equimolar mixture of two 5 or more compounds of claim 47, wherein B, AA, BB and CC are zero, except that AA can be zero or one when R₁ is a hydrogen atom and that CC can be zero or one when R₂ is a hydrogen atom.

49. An approximately equimolar mixture of two 10 or more compounds of claim 48, wherein R₉ is a hydrogen atom and R₁₀ is absent.

50. An approximately equimolar mixture of two or more compounds of claim 49, wherein R₁ and R₂ are independently chosen from the group consisting of S- or R- 15 methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-(napth-2-ylmethylamino)-n-butyl], S- or R-[4-(amino)-n- 20 butyl], S- or R-[sec-butyl], S- or R-(2-amino)ethyl, S- or R-(n-propyl)amine, S- or R-(methylsulfinyl)eth-1-yl, S- or R-[2-(N,N-dimethylamino)ethyl], S- or R-(N, N-diethylamino)ethyl, S- or R-(N,N-diallylamino)ethyl, S- or R-(n-allylamino)ethyl, S- or R-(N,N- 25 dibenzylamino)ethyl, S- or R-(N-benzylamino)ethyl, S- and R-(N,N-di(napth-2-ylmethyl)amino)ethyl, S- and R-(N-(napth-2-ylmethyl)amino)ethyl, S- or R-propionamido, S- or R-(N,N-dimethylamino)propyl, S- or R-(N,N-

diethylamino)propyl, S- or R-(N,N-diallylamino)propyl, S- or R-(N,N-dibenzylamino)propyl, S- or R-(N,N-di(naphth-2-ylmethyl)propionamido, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N,N-diallyl)-3-guanidino-n-propyl], S- or R-
5 [(N,N,N'-triallyl)-3-guanidino-n-propyl], S- or R- [(N,N,N'-trimethyl)-3-(guanidino)-n-propyl], S- or R- [(N,N,N'-triethyl)-3-(guanidino)-n-propyl], S- or R- hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[3-(hydroxy)-n-propyl], S- or R-[iso-propyl], S-
10 or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(ethoxy)benzyl,
15 S- or R-(4-(methoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-(4-hydroxybenzyl), S- or R-(n-butyl), S- or R-(n-propyl), S- or R-[(naphth-2-yl)methyl], AA is zero or one when R₁ is a hydrogen atom, CC is zero or one when R₂ is a hydrogen atom, S- or R-
20 (cyclohexylmethyl), S- or R-[thiolmethyl], S- or R-methylthiol, or when either R₁ or R₂ are taken together with R₃ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

51. An approximately equimolar mixture of two
25 or more compounds of claim 50, wherein R₆ and R₈ are independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl.

52. An approximately equimolar mixture of two or more compounds of claim 48, wherein R₉ is a solid support and R₁₀ is absent.

53. An approximately equimolar mixture of two or more compounds of claim 52, wherein R₉ and R₁₀ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(N-methylamino)-n-butyl], S- or R-[4-(N,N-dimethylamino)-n-butyl], S- or R-[4-(N-methyl-N-ethylamino)-n-butyl], S- or R-[4-(N-ethylamino)-n-butyl], S- or R-[4-(N-methyl-N-ethylamino)-n-butyl], S- or R-[4-(N-allylamino)-n-butyl], S- or R-[4-(N-methyl-N-alkylamino)-n-butyl], S- or R-[4-(N-benzylamino)-n-butyl], S- or R-[4-(N-(napth-2-ylmethylamino)-n-butyl], S- or R-[4-(N-methyl-N-benzylamino)-n-butyl], S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(2-aminoethyl), S- or R-(methylsulfinyl)eth-1-yl, S- or R-acetamido, S- or R-[2-(N,N-dimethylamino)ethyl], S- or R-(N, N-diethylamino)ethyl, S- or R-(N,N-diallylamino)ethyl, S- or R-(n-allylamino)ethyl, S- or R-(N,N-dibenzylamino)ethyl, S- or R-(N-benzylamino)ethyl, S- or R-(N,N-di(naphth-2-ylmethyl)amino)ethyl, S- or R-(N-(napth-2-ylmethyl)amino)ethyl, S- or R-(N-propylamine), S- or R-propionamido, S- or R-(N,N-dimethylamino)propyl, S- or R-(N,N-diethylamino)propyl, S- or R-(N,N-diallyl)propionamido, S- or R-(N,N-dibenzylamino)propyl, S- or R-(N,N-di(naphth-2-ylmethyl)propionamido, S- or R-[3-(N-PMC-guanidino)-n-propyl], S- or R-[(N,N'-diallyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-[(N,N',N''-triallyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-

[(N,N',N'''-trimethyl-N-PMC) -3-(guanidino)-n-propyl], S- or R- [(N,N',N'''-triethyl-N-PMC) -3-(guanidino)-n-propyl], S- or R-hydroxymethyl, S- or R-[1-(hydroxy)ethyl], S-phenyl, S- or R-[3-(hydroxy)-n-propyl], S- or R-[isopropyl], S- or R-[(indol-3-yl)methyl], S- or R-[(N-methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-yl)methyl], S- or R-(4-(ethoxy)benzyl, 10 S- or R-(4-(methoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-[4-hydroxybenzyl], S- or R-[n-butyl], S- or R-(n-propyl), S- or R-[(naphth-2-yl)methyl], S-methyl and CC is one, S- or R-[cyclohexylmethyl], S- or R-[thiolmethyl], AA is zero or one when R₁ is a hydrogen atom, CC is zero or one when R₃ is a hydrogen atom, or 15 when either R₁ or R₂ are taken together with R₃ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

54. An approximately equimolar mixture of two or more compounds of claim 53, wherein R₆ and R₈ are 20 independently methyl, ethyl, allyl, benzyl, or naphth-2-ylmethyl.

55. A method for effecting analgesia in a mammal, which comprises administering an effective amount of a single compound of claim 1 in conjunction with a 25 pharmaceutically-acceptable carrier.

56. A method of claim 55, wherein the single compound has X and Y taken together to form a carbonyl group, B, AA, BB and CC are zero, R₁ is a hydrogen atom,

R₈ is napth-2-ylmethyl, R₁ is S-methyl, R₆ is ethyl, R₃ is S-benzyl, and R₁ and R₂ are each a hydrogen atom.

57. A method of claim 55, wherein the single compound has X and Y taken together to form a carbonyl group, B, AA, BB and CC are zero, R₉ is a hydrogen atom, R₈ is benzyl, R₁ is S-methyl, R₆ is naphth-2-ylmethyl, R₃ is taken in conjunction with either R₁ or R₂ to form an S-pyrrolidine ring and the other of R₁ and R₂ a hydrogen atom.

10 58. A method of effecting a decrease in the postprandial rise in the blood glucose levels of a mammal after ingestion of a carbohydrate load by said mammal, which comprises administering an effective amount of a single compound of claim 1 in conjunction with a 15 pharmaceutically-acceptable carrier.

59. A method of claim 58, wherein the single compound has X and Y taken together to form a carbonyl group, B, AA, BB and CC are zero, R₉ is a hydrogen atom, R₈ is benzyl, R₆ is naphth-2-ylmethyl, R₃ is R-(N-(naphth-20 2-ylmethyl)indol-3-ylmethyl), R₁ and R₂ are each hydrogen, R₁₀ is absent, and R₇ is chosen from the group consisting of S-(4-(N-benzylamino)-n-butyl), R-(4-(N-benzylamino)-n-butyl), S-(3-guanidino)-n-propyl, and R-(3-guanidino)-n-propyl).

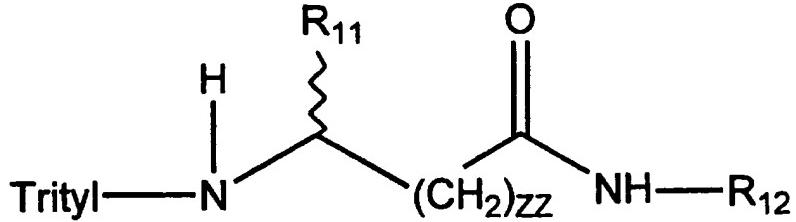
25 60. A method of treating microbial infections in mammals, which comprises administering an effective

amount of a single compound of claim 1 in conjunction with a pharmaceutically-acceptable carrier.

61. A method of claim 60, wherein the single compound has X and Y taken together to form a carbonyl group, B, AA, BB and CC are zero, R₅ is a hydrogen atom, R₆ is benzyl, R₇ is naphth-2-ylmethyl, R₈ is R-(N-(naphth-2-ylmethyl)indol-3-ylmethyl), R₁ and R₂ are each hydrogen, R₁₀ is absent, and R₉ is chosen from the group consisting of S-(4-(N-benzylamino)-n-butyl), R-(4-(N-benzylamino)-n-butyl), S-(3-guanidino)-n-propyl, and R-(3-guanidino)-n-propyl).

62. A method of step-wise N-alkylation of the amide bond of the N-terminal residue of a compound of the Formula (II):

15 (II)



Wherein:

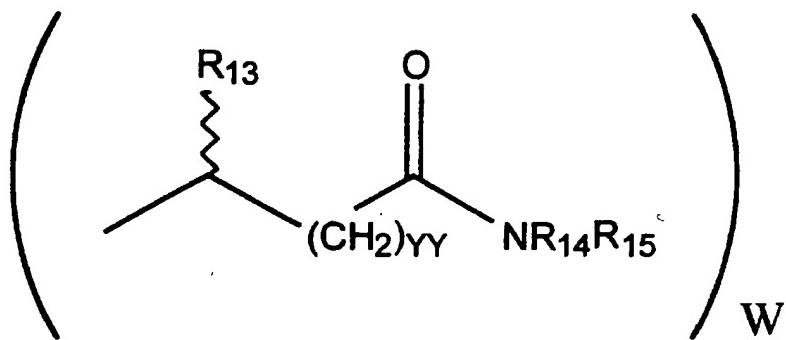
R₁₁ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, 20 a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

ZZ is from zero or five;

And R₁₂ is a solid support or a group of the
Formula (III):

(III)

5



Wherein R₁₄ is a C₁ to C₁₈ substituent group;

Wherein W is 0 to 4;

R₁₃ is independently a hydrogen atom, C₁ to C₁₂,
10 alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted
phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl,
a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅
alkyl heterocycle;

R₁₅ is a solid support (when W is one) or a bond
15 to the preceding methylene group (when W is from two to
four);

YY is from zero to five;

Wherein the compound of the above formula is
a) first reacted under anhydrous conditions in an inert
atmosphere with an excess amount non-nucleophilic base
5 having a pKa between about 18 to about 40; then
b) reacting the resulting anion under anhydrous conditions
in an inert atmosphere in a polar aprotic solvent with an
excess amount of an alkylating agent of the formula

(LG) - Q

10 Wherein LG is leaving group;

Q is a C₁ to C₁₈ substituent group;

and repeating steps a) and b) as necessary to
drive the alkylation to completion;

with the proviso that all previous internal
15 backbone amide bonds have been previously alkylated with
a C₁ to C₁₈ substituent group and, when W is from 2 to 4,
all of the R₁₄ groups are not the same C₁ to C₁₈
substituent group.

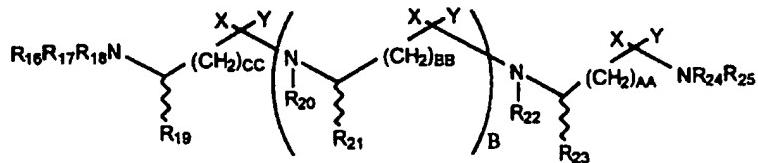
63. A process of claim 62, wherein LG is iodo
20 or bromo and the -CH₂-Q moiety is methyl, ethyl, allyl,
benzyl or naphth-2-ylmethyl.

64. A process of claim 63, wherein R₁₁ and R₁₃ are independently chosen from the group consisting of S- or R-methyl, S- or R-benzyl, a hydrogen atom, S- or R-(but-2-yl), S- or R-[4-(t-butoxycarbonylamino)-n-butyl], 5 S- or R-[4-(amino)-n-butyl], S- or R-[sec-butyl], S- or R-(methylsulfinyl)eth-1-yl, S- or R-[3-(guanidino)-n-propyl], S- or R-[(N-PMC)-3-(guanidino)-n-propyl], S- or R-(t-butoxy)methyl, S- or R-[2-(t-butoxy)ethyl], S-phenyl, S- or R-[2-(t-butoxycarbonyl)ethyl], S- or R-[iso-10 propyl], S- or R-[(N-(t-butoxycarbonyl)indol-3-yl)methyl], S- or R-[4-hydroxybenzyl], S- or R-[(4-(t-butoxy))benzyl], S- or R-[n-propyl], S- or R-(n-butyl), S- or R-[(naphth-2-yl)methyl], S- or R-(2-carboxyethyl), S- or R-(cyclohexylmethyl), S-[(4-15 methoxybenzylthio)methyl], S- or R-[(4-methylbenzylthio)methyl], S- or R-thiomethyl, S- or R-[4-(N-methyl-(N-(t-butoxycarbonly))amino)-n-butyl], S- or R-[4-(N-ethyl-(N-(t-butoxycarbonly))amino)-n-butyl], S- or R-[4-(N-allyl-(N-(t-butoxycarbonly))amino)-n-butyl], 20 S- or R-[4-(N-benzyl-(N-(t-butoxycarbonly))amino)-n-butyl], S- or R-[4-(N-(naphth-2-yl)-(N-(t-butoxycarbonly))amino)-n-butyl], S- or R-acetamino, S- or R-[2-(N,N-dimethyl)acetamido], S- or R-(N, N-diethyl)acetamido, S- or R-(N,N-diallyl)acetamido, S- or 25 R-(n-allyl)acetamido, S- or R-(N,N-dibenzyl)acetamido, S- or R-(N-benzyl)acetamido, S- or R-(N,N-di(naphth-2-ylmethyl))acetamido, S- or R-(N-(naphth-2-ylmethyl))acetamido, S- or R-(n-propyl)amine, S- or R-propionamido, S- or R-(N,N-diethyl)propionamido, S- or R-30 (N,N-diallyl)propionamido, S- or R-(N,N-dibenzyl)propionamido, S- or R-(N,N-di(naphth-2-

ylmethyl)propionamido, S- or R-[(N,N'-diallyl-N-PMC)-3-guanidino-n-propyl], S- or R-[(N,N',N''-trimethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-[(N,N',N''-triethyl-N-PMC)-3-(guanidino)-n-propyl], S- or R-[(N,N',N''-trialkyl-5-N-PMC)-3-guanidino-n-propyl], S- or R-[(indol-3-yl)methyl], S- or R-[(N-(methyl)indol-3-yl)methyl], S- or R-[(N-(ethyl)indol-3-yl)methyl], S- or R-[(N-(allyl)indol-3-yl)methyl], S- or R-[(N-(benzyl)indol-3-yl)methyl], S- or R-[(N-(naphth-2-ylmethyl)indol-3-10-yl)methyl], S- or R-(4-(ethoxy)benzyl, S- or R-(4-(methoxy)benzyl, S- or R-(4-(allyloxy)benzyl, S- or R-(4-(benzoxy)benzyl, S- or R-(4-(naphthl-2-ylmethoxy)benzyl, ZZ is one or zero when R₁₁ is a hydrogen atom, YY is one or zero when R₁₃ is a hydrogen atom, or 15 when either R₁₃ is taken together with R₁₄ to form an S- or R-pyrrolidine or S-[4-(hydroxy)pyrrolidine].

65. A method of synthesizing and testing for biological activity a library of an approximately equimolar amount of compounds of the following Formula (IV):

20 (IV)



Wherein in the above Formula (IV):

R₁₉, R₂₁ and R₂₃ independently are a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl,

substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₂₅ is a hydrogen atom or a solid support;

5 R₂₀, R₂₂ and R₂₄ are independently a C₁ to C₁₈ substituent group;

AA, BB and CC are independently 0 to 5;

B is from 0 to 3;

X and Y are taken together to form a carbonyl 10 group or are separate and are each a hydrogen atom;

R₁₆, R₁₇, and R₁₈ independently are a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted 15 alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle; R₁₆ is optionally present as a C₁ to C₁₈ substituent group when R₁ and R₂ are other than a hydrogen atom or an amino protecting group;

Wherein said library of compounds is composed 20 of SL physically separate sublibraries; wherein SL is equal to (2B+4);

Further wherein each sublibrary is composed of physically separate mixtures, wherein the number of said

mixtures is equal to the number of different substituents incorporated at R_{fix} , which R_{fix} can be any one of R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , or R_{24} in the above Formula IV;

Wherein the compounds of the above Formula IV
5 are synthesized and tested as follows:

(a) For each sublibrary SL, choosing R_{fix} from R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , or R_{24} ;

(b) Dividing a solid support into approximately equal separate portions with the number of 10 portions equal to the number of substituents to be incorporated at R_{23} , and couple each physically separate portions of solid support to one of the monomers containing a single substituent at R_{23} , then mixing all of said physically separate portions;

15 (c) Dividing the mixed solid support from step (a) into approximately equal separate portions in a number equal to the number of different substituents to be incorporated at R_{24} by alkylation, alkylating each physically separate solid support mixtures with one alkyl 20 group, then mixing said resins;

(d) When B is 1 through 3, dividing each of said solid support portions into a number of approximately equal separate portions, said number equal to the number of substituents at R_{21} , coupling one said 25 monomer containing a single substituent R_{23} to each separate solid support portion then mixing said portions;

(e) When B is 1 to 3, separating said mixture of solid support portions into a number of approximately equal physically-separate portions, said number equal to the number of alkyl substituents at R₂₀, 5 alkylating each physically separate portion with one such alkylating agent, and mixing all the resultant solid support portions;

(f) Optionally repeating steps (d) and (e) one or two times when B is two or three, 10 respectively;

(g) Dividing the mixture of solid support portions from either step (c), (e), or step (f) into approximately equal separate portions equal to the number of substituents to be placed at R₁₉, coupling one such 15 monomer containing a single R₁₉ to each physically separate solid support portion, and mixing said portions;

(h) Dividing the mixture of portions from step (g) into a number of approximately equal separate portions, said number equal to the number of alkyl substituents at R₂₂, to be utilized, alkylating each said 20 separate portion with a single alkyl group R₂₃;

(i) Optionally adding R₁₇ and/or R₁₈ by reductive alkylation;

(j) Optionally adding the quaternary 25 substituent R₁₆;

(k) Optionally reducing the interior amides, thus converting X and Y taken together are a carbonyl oxygen to wherein each X and Y is a hydrogen atom;

5 (l) Cleaving said molecules from the solid support;

(m) Testing each portion of each SL sublibraries in the appropriate biological screen or screens; and determining from the results of said screens
10 which substituent at R_{fix} is the best.

(n) Optionally synthesizing the molecule of Formula (I) containing the best (R_{fix}) substituent at R_{19} , R_{20} , R_{21} , R_{22} , R_{23} , or R_{24} ;

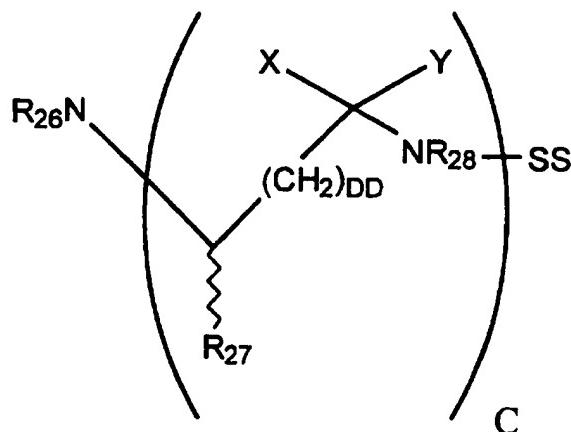
With the proviso that for each sublibrary SL
15 the first solid support mixing step immediately following the introduction of R_{fix} is omitted;

Further wherein:

(1) each coupling step in the above series of steps ((b), (d), (f) and (g)) involves a substrate of
20 the Formula (V):

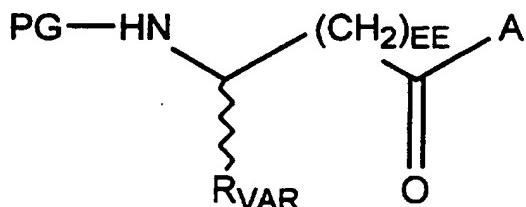
135

(V)



With an excess of an active acylating form of
the monomer of the Formula (VI) :

(VI)



5

Wherein in the above Formulas (V) and (VI) :

SS is a solid support;

R_{26} are two hydrogen atoms each bound to the
nitrogen atom;

10 R_{28} is a C_1 to C_{18} substituent group;

R_{27} is independently a hydrogen atom, C_1 to C_{12}
alkyl, C_1 to C_{12} substituted alkyl, phenyl, substituted
phenyl, C_7 to C_{16} alkylaryl, C_7 to C_{16} substituted alkylaryl,

a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R_{VAR} can be the same or different as R₂₇, and is chosen from the same group of substituents as R₂₇;

5 DD and EE are independently 0 to 5;

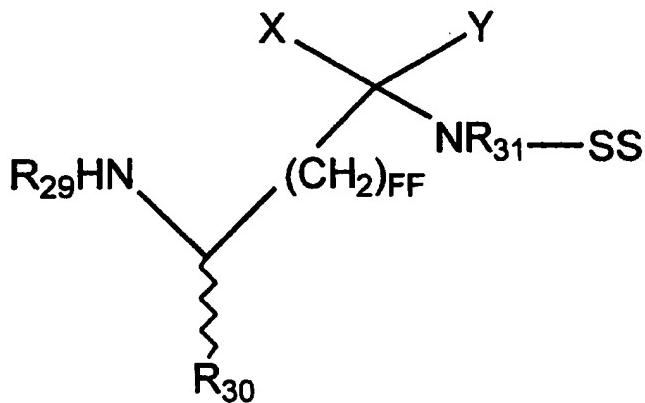
X and Y are either taken together to form a carbonyl oxygen;

PG is an amino protecting group other than trityl;

10 A is a group, when taken with the preceding carbonyl group; that forms an active acylating agent; and C is from 0 to 4;

(2) Each alkylating step in the above steps (c), (e), (f) and (h) requires reacting a substrate
15 of the Formula (VII):

(VII)



With an excess of an alkylating agent of the
Formula (VIII):

(VIII)

(LG) - Q

Under anhydrous conditions, and an inert
5 atmosphere in a polar, aprotic solvent;

Wherein in the above Formulas (VII) and (VIII):

LG is a leaving group under the conditions of
the alkylation;

Q is a C₁ to C₁₈ substituent group;

10 FF is 0 to 5;

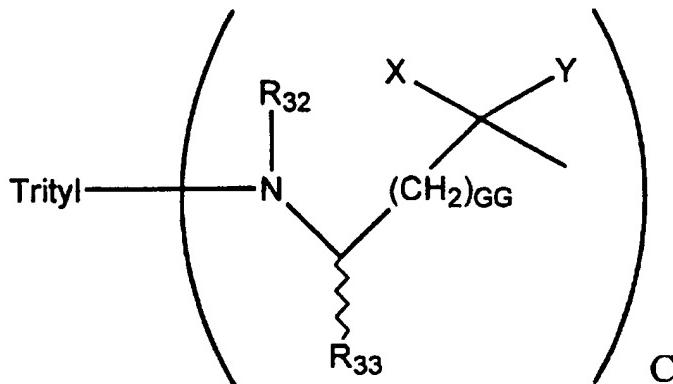
X and Y are taken together to form a carbonyl
oxygen;

R₃₁ is a hydrogen atom when R₂₉ is a trityl group
or it is a C₁ to C₁₈ substituent group;

15 R₃₀ is independently a hydrogen atom, C₁ to C₁₂
alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted
phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl,
a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅
alkyl heterocycle;

20 R₂₉ is a trityl group when R₃₁ is a hydrogen atom
or is a group of the Formula (IX):

(IX)



Wherein in the above Formula (IX) :

X and Y are as X and Y above;

GG is 0 to 5;

5 C is from 1 to 4;

R₃₃ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl,

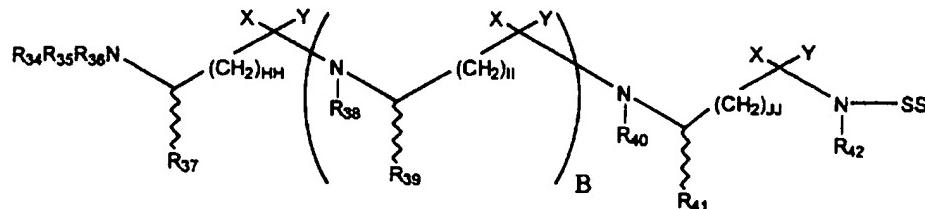
a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅

10 alkyl heterocycle;

R₃₂ is a hydrogen atom if R₃₂ is bonded to the N-terminal amino group or otherwise it is a C₁ to C₁₈ substituent wherein one such C₁ to C₁₈ substituent differs from the other substituents;

15 (3) Optimal reductive alkylation of the N-terminal nitrogen group as described above in step (i) of a compound of the Formula (X) :

(X)



Under mildly acidic conditions with a ketone or aldehyde containing the R₃₄ and/or R₃₅ groups followed by
5 the treatment of a reducing agent;

Wherein in the above Formula (X) :

X and Y are taken together to form a carbonyl oxygen;

HH, II and JJ are independently 0 to 5;

10 B is from 0 to 3;

R₃₉, R₄₁ and R₃₇ are the same or different and are chosen from the group consisting of independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;
15

R₄₀ and R₄₂ are different and are a C₁ to C₁₈ substituent group;

R₃₄ or R₃₅ when B is zero, is optionally one or
20 two hydrogen atoms attached to the nitrogen atom, or is a optionally one or more, same or different groups, chosen from the group consisting of a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted

alkyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₃₆ is a hydrogen atom or a bond to a R₃₄ or R₃₅ group before the reduction occurs and when B is from 1 to 3; R₃₈ is a C₁ to C₁₈ substituent group different from at least one other R₄₀, R₄₂, or R₃₈ group;

(4) Optional reduction of the amide bonds as described above in step (j) of a compound of Formula 10 (X) wherein X and Y are taken together to form a carbonyl group, before or after it is cleaved from the solid support, using a boron reducing agent; and

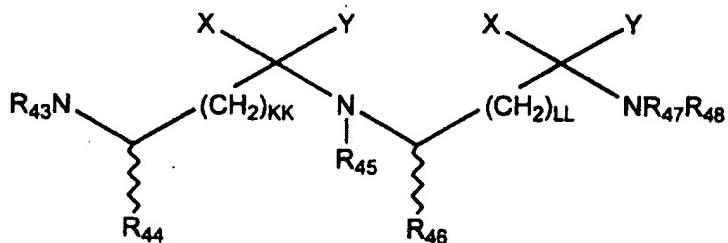
(5) Optional quaternization of the terminal amino groups with excess amount of an alkylating 15 agent of above of the formula:

(LG) - Q

Where (LG) and Q are as defined above in a polar, aprotic solvent.

66. A method for the iterative synthesis and 20 screening of a library of an approximately equimolar amount of compounds of the Formula (XI):

(XI)



Wherein in the above Formula (XI) :

R_{48} is a hydrogen atom or a solid support;

5 R_{45} and R_{47} are different and are each a C₁ to C₁₈ substituent group;

KK and LL are independently 0 to 5;

10 R_{44} and R_{46} are independently chosen from the group consisting of independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

15 X and Y are either taken together to form a carbonyl group or are separate and are each a hydrogen atom;

- R_{43} is one or two hydrogen atoms, or groups of the formula R_a, R_b and R_c, wherein R_a and R_b independently are a hydrogen atom, an amino protecting group, C₁ to C₁₂

acyl, C₃ to C₁₀ cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle; R_c is 5 optionally present as a C₁ to C₁₈ substituent group when R_a and R_b are other than a hydrogen atom or an amino protecting group; and

Wherein the method comprises:

(a) Splitting a solid support into a 10 number of approximately equal, separate portions, the number of said portions being equal to the number of monomers containing different substituent groups at R₄₆;

(b) Coupling each different monomer containing one of the number of substituent groups at R₄₆ 15 to a separate portion of the solid support;

(c) Mixing all of the separate portions of solid support;

(d) Splitting the solid support mixture into approximately equal, separate portions, the number 20 of portions equal to the number of different substituents to be added at R₄₇;

(e) Alkyinating each separate portion of solid support with a single alkylating agent, each agent containing a unique alkyl group at R₄₇, thus adding a 25 single alkyl group at R₄₇ to the plurality of the

compounds bonded to each separate portion of solid support;

(f) Mixing all of the separate portions of solid support;

5

(g) Splitting the solid support mixture into a number of approximately equal, separate portions, the number of said portions equal to the number of different substituents to be added at R₄₄;

10

(h) Coupling each monomer containing a single substituent group at R₄₄ to a separate portion of the solid support, thus coupling a single different monomer to the plurality of the compounds bonded to each separate portion of the resin;

15

(i) Splitting each of the separate portions of solid support into a number of approximately equal physically-separate portions, wherein the number of portions is equal to the number of different substituents to be added by alkylation at R₄₅;

20

(j) Alkylating each separate portion of solid support with a separate alkylating agent containing a single different R₄₅ group, thus adding a single different alkyl group at R₄₅ to the plurality of the compound bonded to each separate portion of the resin;

25

(k) Cleaving the generated compound mixtures of Formula (XI) from each separate portion of

solid support and testing each separate mixture from each separate portion of solid support in the appropriate biological screen or screens, and determining from the results of said screens which mixture contains the best 5 combination of substituents R_{44} and R_{45} ;

(l) Repeating steps (a) through (e), wherein the substituents at R_{46} and R_{47} are the same used in said original steps (a) through (e);

10 (m) Coupling the monomer containing the most active R_{44} substituent to each of the separate portions of resin from step (l);

(n) Alkyllating each of the portions from step (m) with the best alkyl group at R_{45} determined in step (k);

15 (o) Cleaving each separate mixture of compounds of the above Formula (XI) from the solid support, testing each separate mixture of compounds in the same biological screens as in step (k), and determining the most active substituent at R_{47} in those 20 screens;

(p) Repeating steps (a) and (b), wherein the same group of monomers containing the various substituent R_{46} are used as in the original step (a);

25 (q) Alkyllating each separate resin portion from step (p) with an alkylating agent placing

the best alkyl group at R₄, as such alkyl group was determined in step (o);

(r) Coupling to each separate portion of resin the monomer containing the best R₄₄ substituent as 5 such substituent was determined in step (k);

(s) Alkylating each separate portion of resin with a group that was the best alkyl group R₄₅ as such group determined in step (k);

(t) Cleaving each separate compound from 10 the solid support, and testing each separate mixture of compound separately in the same screens as in steps (o) and (k) in order to determine the best substituents at R₄₆;

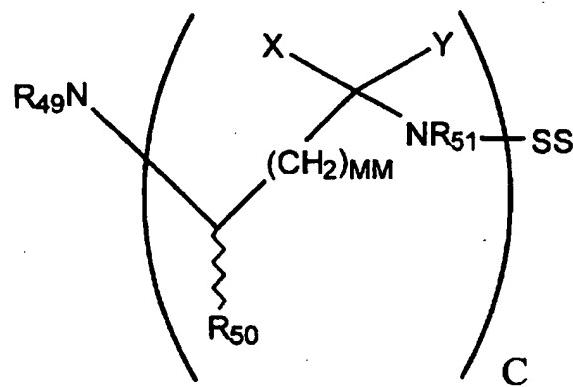
(aa) Optionally reductively alkylating and 15 quaternizing the N-terminal amino group (R₄₃) , either before or after cleavage of the compound from the solid support; and

(bb) Optionally reducing the interior amide groups before or after cleavage of the compound 20 from the solid support such that X and Y in Formula (XI) are each a hydrogen atom;

Further wherein:

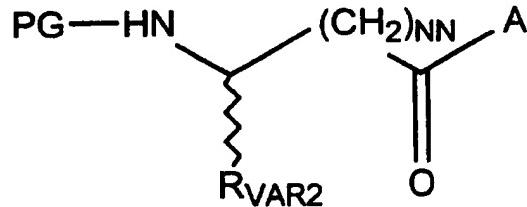
(1) each of the above coupling steps (b),
 (h), (l), (m), (p) or (r), involves a substrate of the
 Formula (XII) :

(XII)



5 With an excess of an active acylating form of
 the monomer of the Formula (XIII) :

(XIII)



Wherein in the above Formulas (XII) and (XIII) :

SS is a solid support;

R₄₉ is two hydrogen atoms;

R₅₁ is a C₁ to C₁₈ substituent group

R₅₀ is independently a hydrogen atom, C₁ to C₁₂, alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R_{VAR2} can be the same or different as R₅₀ and is chosen from the same group of substituents as R₅₀;

MM and NN are independently 0 to 5;

X and Y are either taken together to form a carbonyl group or are separate and are each a hydrogen atom;

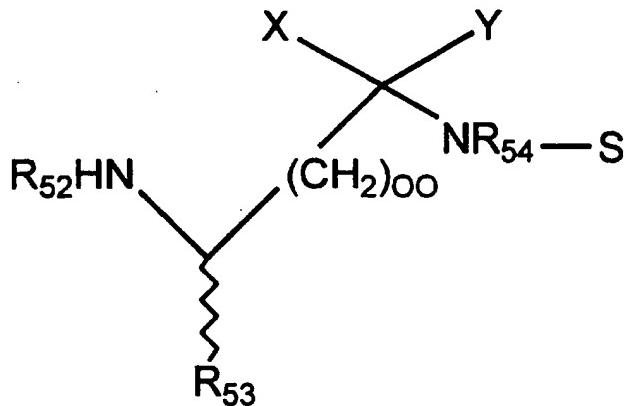
PG is an amino protecting group other than trityl;

A is a group, when taken with the preceding carbonyl group; that forms an active acylating agent; and

C is 0 or 1;

(2) Each of the above alkylating steps (e), (j), (l), (m), (q) and (s), requires reacting a substrate of the Formula (XIV):

(XIV)



With an excess of an alkylating agent of the
Formula (XV) :

5 (XV)

(LG) - Q

Under anhydrous conditions, and an inert
atmosphere in a polar, aprotic solvent;

Wherein in the above Formulas (XIV) and (V) :

LG is a leaving group under the conditions of
10 the alkylation;

Q is a C₁ to C₁₈ substituent group;

OO is 0 to 5;

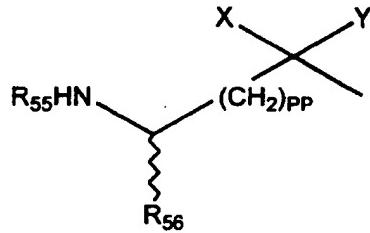
X and Y are taken together to form a carbonyl
group; or are separate and are each a hydrogen atom;

15 R₅₄ is a hydrogen atom or it is a C₁ to C₁₈
substituent group;

R₅₃ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

R₅₂ is a trityl group if R₅₄ is a hydrogen atom or is a group of the Formula (XVI) :

(XVI)



10

Wherein in the above Formula (XVI) :

X and Y are as X and Y above;

PP is 0 to 5;

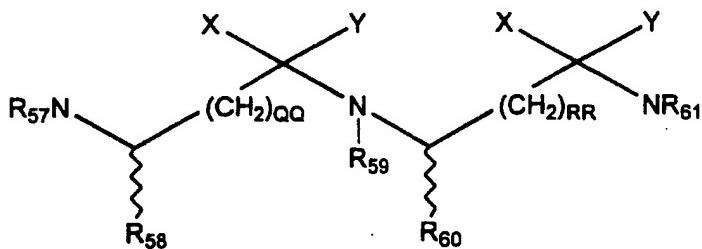
R₅₅ is a trityl group;

R₅₆ is independently a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle; and

20

(3) Optional reductive alkylation of the N-terminal nitrogen group of a compound of the Formula (XVII) :

(XVII)



Under mildly acidic conditions with a ketone or aldehyde containing the R₁ and/or R₂ groups followed by
5 treatment with a reducing agent;

Wherein in the above Formula (XVII):

X and Y are taken together to form a carbonyl group;

QQ and RR are independently 0 to 5;

10 R₅₈ and R₆₀ are the same or different and are chosen from the group consisting of independently of a hydrogen atom, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, phenyl, substituted phenyl, C₁ to C₁₆ alkylaryl, C₁ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a 15 substituted C₆ to C₁₅ alkyl heterocycle;

R₅₉ and R₆₁ are the same or different and are a C₁ to C₁₈ substituent group;

15 R₅₇ is either two hydrogen atoms attached to the nitrogen atom, or is a single hydrogen atom and 20 another group bonded to the nitrogen atom which group is selected from the group consisting of a hydrogen atom, an amino protecting group, C₁ to C₁₂ acyl, C₃ to C₁₀

cycloalkyl, C₃ to C₆ heterocycle, C₁ to C₁₂ alkyl, C₁ to C₁₂ substituted alkyl, C₇ to C₁₆ alkylaryl, C₇ to C₁₆ substituted alkylaryl, a C₆ to C₁₅ alkyl heterocycle, or a substituted C₆ to C₁₅ alkyl heterocycle;

- 5 (4) Optional reduction of the amide bonds of a compound of Formula (XI), before or after it is cleaved from the solid support, using a boron-based reducing agent, such as borane, sodium borohydride, and the like.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB97/00349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/53, 33/566, 33/543
US CL : 435/7.1; 436/501

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1; 436/501, 518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, CAPLUS, MEDLINE
search terms:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DORNER, B. et al. Generation of Peralkylated Peptidomimetic Combinatorial Libraries. Methods in Molecular and Cellular Biology. 1996, Vol. 6, pages 17-22, entire document.	1-8,32-34,40 62-64
Y	OSTRESH, J.M. et al. "Libraries from Libraries": Chemical Transformation of Combinatorial Libraries to Extend the Range and Repertoire of Chemical Diversity. Proc. Natl. Acad. Sci. USA. November 1994, Vol. 91, No. 23 pages 11138-11142, entire document.	----- 9-31,35-39, 41-54,65,66
Y	HOUGHTEN, R.A. et al. Libraries from Libraries: The generation of Peptidomimetic Combinatorial Diversities. Proc. Eur. Pept. Symp. 23. 1994, pages 459-460, entire document.	9-31,35-39, 41-54,65,66
Y		55-61

Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:		
A document defining the general state of the art which is not considered to be of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*&*	document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

16 JUNE 1997

Date of mailing of the international search report

25 JUL 1997

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